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THE EFFECT OF WIDE VARIATIONS IN POTASSIUM AND SODIUM INTAKE IN ASTHMATIC CHILDREN

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CLINICAL investigations of potassium therapy in allergic disorders have led to contradictory results and a wide divergence of opinion regarding its efficacy. Excellent results in the treatment of certain disorders with potassium salts have been reported by Rusk and his coworkers,[1-3] Bloom,[4] Bloom and Grauman,[5] Abt,[6] Parker,[7] and Smith and Steffensen.[8] Essentially negative results in the treatment of the same disorders with the same medication have been reported by Cohen,[9] Engelsher,[10] Miller and Piness,[11] Harsh and Donovan,[12] Harley,[13] Rubin and his associates,[14] Spain, Westcott, and Galliard,[15] and Furstenberg and Gay.[16] Relatively few of the investigators reported on the results of potassium therapy in asthma, and their reports were confusing. Rusk, Weichselbaum, and Somogyi[2] reported a decrease in plasma potassium and a reduction in asthmatic symptoms; Bloom and Grauman[5] reported poor results in chronic asthma but good results in "hay fever and pollen asthma"; while Parker[7] reported good results in both asthma and hay fever. The few others who reported asthma cases observed little or no benefit from the therapy.

The most obvious criticism of these investigations is that the total amount of potassium ingested by the patients was not controlled. The doses of potassium salts were carefully measured, but the amounts of potassium ingested by the patients in their diets were unknown or not reported. This means that the total intake of potassium by these patients must have varied considerably, for, as we have pointed out else-

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where,[12] the variations in potassium intake due to variations in diet are probably much greater than those effected by the usual doses of potassium salts.

The importance of total intake of potassium and sodium on the efficacy of potassium therapy in asthma is well illustrated in the experiments reported by Stoesser and Cook.[17] These authors maintained children with intractable asthma, in an air-conditioned room, on diets extremely low in potassium and sodium, and observed that, under these conditions, potassium chloride caused the cessation of mild attacks of asthma. The more severe asthma continued while the salt was being taken but ceased promptly when it was discontinued. The potassium chloride medication was much less effective, however, than the drastic reduction of sodium. It should be noted that the regimen used by Stoesser and Cook would not be practicable in the treatment of outpatients, that the diet was deficient in protein and that the authors made no therapeutic claims on the basis of these experiments.

It should also be remembered that Stoesser and Cook's patients were not exposed to wide variations in temperature, relative humidity, or acid-base content of food. Kern[18] has pointed out that meteorologic conditions, acid-base metabolism, and other variables affecting the water balance also affect the severity of asthmatic symptoms. This being true, it is entirely reasonable to suspect that these variables would also affect the response of asthmatic symptoms to potassium therapy and that they might account in part for the wide divergence in the results obtained with this therapy.

Another variable which probably contributed to the divergence in the reported results of potassium therapy is the extent to which the different investigators minimized the effect of subjective factors. This would be true particularly if a large proportion of the reported cases were mild, but it is unlikely that subjective factors alone could account for such widely divergent reports as "complete remission of symptoms" and "little or no effect" in any considerable number of severe cases treated with the same medication.

The doubt that subjective factors alone could account for the wide divergence in reported results, together with our conviction that potassium therapy in allergy has not been adequately tested, made it seem important to us to test the efficacy of this salt under such conditions that the complicating factors mentioned above could be controlled or their effects evaluated. We wanted, specifically, to test the effect of the widest variations in total potassium and sodium intake practicable in the treatment of outpatients on the allergic symptoms of a sizable group of persons suffering from allergy of varied duration and severity. These persons would eat the same foods and be exposed to the same known variations in meteorologic conditions and air-borne allergens.

Through the friendly cooperation of the management and children of a local children's home, we were able to satisfy most of these conditions and to test the effect of wide variations in the total intake of potassium and sodium on the symptoms of twelve children who were in the home because of recurrent asthmatic attacks. These children, eleven boys and one girl, ranged in age from 5 to 13 years and included representatives of several racial stocks. There was also considerable variation in the duration and severity of their asthma. All had been skin tested and were known to be sensitive to certain foods or air-borne allergens. The food sensitivities of the group, however, were limited sufficiently to permit the construction of well-balanced, diversified menus from foods to which none of the children was sensitive. Violent exercise was forbidden, and games requiring only moderate exercise were provided for the play periods when the children were not in school. All of the children were volunteers for the rather onerous duties and restrictions of the study, and all were remarkably friendly and cooperative throughout the entire period.

The children were attended by the regular staff of the home and by a special nurse experienced in the handling of asthmatic children. It was the nurse's duty to supervise the preparation, weighing, and serving of the children's food and the collection of urine specimens. She gave the prescribed doses of potassium and sodium chlorides and kept a careful record of the incidence, severity, and duration of asthmatic attacks, together with the medication necessary for the relief of symptoms. The children also were observed daily by one of us, and the nurse's estimate of the severity of all asthmatic attacks was carefully checked.

The study began with a preliminary week during which the routine procedures of the study were established. The children ate the foods regularly served to them by the institution, and no control of their potassium and sodium intake was attempted. The incidence, duration, and severity of asthmatic attacks were described by the staff as "ordinary," so that this week may serve as a control on the effect of the usual intake of potassium and sodium on the asthmatic attacks of these children.

During the second, fourth, and sixth weeks, the diet was selected from the permitted foods to provide a maximum intake of potassium. Weighed servings of these foods were given to each child, and the amount left uneaten was deducted. Sodium was restricted to the amount occurring naturally in the selected foods, and each child was given an individual salt-cellar containing a weighed amount of potassium chloride to be used as seasoning. In addition, each child received a solution of potassium chloride equivalent to 7.5 Gm. of the salt per day. This solution was given in equal doses after each meal. There were some complaints of nausea and epigastric pain, but there were no severe gastrointestinal upsets. Incidentally, it might be mentioned that there were

noticeable evidences of euphoria on the high-potassium diets, so that members of the staff, who did not know what medication was being given, remarked that the children were more cheerful and responsive during weeks on the high-potassium diets than during weeks when the diet was low in potassium and high in sodium.

During the third and fifth weeks of the study the potassium was restricted to the amount occurring naturally in the diet, which was selected from among the permitted foods to give a high intake of sodium. The sodium in the food was supplemented by weighed seasonings of sodium chloride and by the ingestion of sodium chloride solution equivalent to 6 Gm. of the salt per day. The menus for each day were planned in advance by an experienced dietitian who was familiar with the dietary requirements of the experiment and the food allergies of the children. There was also splendid cooperation in the preparation and serving of the food.

TABLE I

DAILY INGESTION OF POTASSIUM AND SODIUM (AS CHLORIDES) BY ASTHMATIC CHILDREN ON DIETS ALTERNATELY HIGH IN POTASSIUM AND SODIUM

WEEK	DIET	POTASSIUM CHLORIDE (GM.)		SODIUM CHLORIDE (GM.)	
		RANGE	MEAN	RANGE	MEAN
1	Control				
2	High K	14.7-18.7	16.8	3.3- 7.1	4.8
3	High Na	3.4- 4.6	4.0	9.7-12.4	11.2
4	High K	11.6-12.8	12.2	3.5- 5.9	4.3
5	High Na	3.4- 6.3	5.2	7.9-12.7	10.9
6	High K	16.4-20.8	18.1	4.3- 6.6	5.1

The range and mean of the daily intake of potassium and sodium during the weeks on these experimental diets are shown in Table I. These values have been expressed as chlorides in order to facilitate comparison with reported intakes of these salts. It should be noted that the mean daily intake of potassium varied approximately 200 to 350 per cent and that more than half of the total intake was ingested in the food during two of the weeks. The variations in potassium intake from week to week on the high potassium diets were effected entirely by selection of foods.

The effect of these wide variations in sodium and potassium intake on the incidence, duration, and severity of asthmatic symptoms is shown in Table II. The incidence and duration are obvious from the table, but an explanation of our method of rating severity may be in order. We rated any patent evidence of asthma which did not require medication and did not curtail the activities of the child as 1. Any attack severe enough to require medication, but which responded promptly and did not require bed rest, was rated as 2. Any prolonged attack which required bed rest, but which could be controlled fairly well by

TABLE II

EFFECT OF DIETS HIGH IN POTASSIUM OR SODIUM ON INCIDENCE, DURATION, AND SEVERITY OF ASTHMATIC ATTACKS

PATIENT	MEAN DAILY INGESTION OF POTASSIUM AND SODIUM (GM.)																																							
	CONTROL							KCl – 16.8 NaCl – 4.8							KCl – 4.0 NaCl – 11.2							KCl – 12.2 NaCl – 4.3							KCl – 5.2 NaCl – 10.9							KCl – 18.1 NaCl – 5.1						
W. B.									2											3								1			1	1									
A. F.				4	3	3	3	1				3	1				3	3	2	1	1	1				1		4	3	2	1	2	4		3	3	3	3	3	3
E. F.											2											1	1	1	2	1	1	1			3	2	1	1				1		
A. G.										1																														
L. H.																																							
H. K.																																							
R. L.				4	3	3	1				3	1				3	1										1			1				1	4	3	3	3	
A. N.							3	2								1						2	1	1	1	1	1	2			3	1	1	1						
H. S.									4	2	3	1		3	1																									
A. S.											1	2	2			1	2	3	4		3	4	4	2																
H. U.																																							
E. V.	1	2						2			4	1	2	1			2	1						2	1			1	3	1				3						
No. of days of asthma	11							21						16						21						24						13						
Units of asthma*	30							41						34						33						42						36						
Week	First							Second						Third						Fourth						Fifth						Sixth						

*Number of days of asthma × mean severity.
Severity of asthma rated 1 to 4.

means of an epinephrine vaporizer, was rated as 3. Finally, any attack which could not be controlled by means of the vaporizer alone was rated as 4.

Three of the children, L. E., H. K., and H. U., had no asthma during the study period, and one child, A. G., had only one mild attack. Some of the other children, however, had more or less severe asthma during almost all of the period.

TABLE III

RELATION OF ASTHMA TO MEAN DAILY INGESTION OF POTASSIUM AND SODIUM CALCULATED AS CHLORIDES

UNITS OF ASTHMA	KCl AND NaCl (GM.)	CONTROL	HIGH KCl	HIGH NaCl	HIGH KCl	HIGH NaCl	HIGH KCl
60	20						
							- - - - -
			- - - - -				
45	15						
			———			———	
					- - - - -		———
				- o - o -	———	- o - o -	
30	10	———					
15	5		- o - o -		- o - o -	- - - - -	- o - o -
				- - - - -			
Week		First	Second	Third	Fourth	Fifth	Sixth

———, Units of asthma (days of asthma × mean severity).
- - - - -, Mean daily ingestion of KCl in grams.
-o-o-, Mean daily ingestion of NaCl in grams.

The relation of the amount of asthma each week, calculated as the product of the total number of days of asthma and the mean severity, to the mean daily ingestion of potassium and sodium chlorides of that week is shown in Table III. It is evident from this table that there was little variation in the amount of asthma from week to week except for an increase in the second week, on high potassium, and in the fifth week, on high sodium.

The relatively poor results on the first high-potassium diet were due probably to the effect of meteorologic conditions. San Diego, as a rule, is not subjected to wide variations in weather conditions, but this week was an exception, as is shown in Table IV. This was a warm, dry, sunshiny period with a high percentage of east, or land, wind. During this week many private patients also complained of exacerbation of their allergic symptoms. This increase of symptoms was not due to an

an increase in air-borne molds or pollens, since the mold count dropped sharply and there was no rise in pollen count as compared to the previous week. Neither was it due to variations in temperature or barometric pressure. There was a decided drop in relative humidity, however, with values as low as 13 per cent, due to a large increase in the percentage of warm, dry, land winds, and a concomitant increase in the relative percentage of sunshine. We are unable to account for the increase of asthma in the fifth week, on high sodium, except on the basis of the large amount of sodium ingested. However, there was no similar increase in the third week when the ingestion of sodium was also high.

TABLE IV

RELATION OF ASTHMA (DAYS OF ASTHMA TIMES MEAN SEVERITY) TO POLLEN COUNT, MOLD COUNT, AND METEOROLOGIC DATA

	WEEKS					
	1 (CONTROL)	2 (KCl)	3 (NaCl)	4 (KCl)	5 (NaCl)	6 (KCl)
Pollen (mean daily) (24 hr., 1 sq. in.)	16	15	13	8	13	6
Mold spores (mean daily) (24 hr., 1 sq. in.)	111	27	83	55	26	20
Asthma (units)	30	41	34	33	42	36
Mean relative humidity (%)	66	47	72	71	62	84
Wind from land (%)	25	51	42	40	25	37
Mean sunshine (%)	77	97	82	76	85	37
Mean temperature (total range 53-86° F.)	64	66	60	65	63	60
Mean barometric pressure (total range 756-768)	763	763	763	763	764	763

TABLE V

EFFECT OF DIETS ALTERNATELY HIGH IN POTASSIUM AND SODIUM ON pH, FREE ACIDITY, AND FORMOL TITRATION OF URINES OF ASTHMATIC CHILDREN

SPECIMENS	DATA	HIGH POTASSIUM		HIGH SODIUM	
		RANGE	MEAN	RANGE	MEAN
First 24-hour specimens on each diet	Volume	360 – 2,200	1,310	330 – 1,900	1,030
	pH	5.0 – 8.3	6.2	4.8 – 7.1	5.8
	Free acidity	*00 – 61	19	10 – 69	35
	Formol titration	11 – 158	32	19 – 58	31
Subsequent first morning specimens	pH	4.7 – 6.2	5.4	4.8 – 6.9	5.7
	Free acidity	22 – 70	40	12 – 56	32
	Formol titration	17 – 59	37	15 – 55	31

*At phenolphthalein end point without NaOH; ammoniacal decomposition.
Free acidity and formol titration: c.c. of N/10 NaOH per 100 c.c. of urine.

The amount of acid-ash foods ingested on the high-potassium diets was appreciably greater than the amount ingested on the high-sodium diets. The amount of acid excreted during the first twenty-four hours on the high-potassium diets, however, was not appreciably greater than the amount excreted during the first twenty-four hours on the high-

sodium diets. This is shown in Table V, which summarizes the values obtained for pH, free acidity, and formol titration of the first twenty-four-hour urine specimens and subsequent first morning specimens, on each of the diets. The free acidities and formol titrations of the twenty-four-hour specimens were different, but the difference in concentration of total acid is balanced by the difference in volume. It should be noted also that there was a marked difference between the concentrations of acid in the first twenty-four hours and subsequent first morning specimens on the high-potassium diets, whereas the difference in the concentrations of acid in similar specimens on the high-sodium diets was negligible.

SUMMARY

1. The effect of wide variations in total ingestion of potassium and sodium on the symptoms of twelve asthmatic children has been observed under conditions that permitted the control or evaluation of such complicating factors as variations in diet, meteorologic conditions, and amount of air-borne allergens.

2. There is some indication that a high-sodium intake increased the amount of asthma of the group, but a high-potassium intake had little or no effect.

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THE IMMUNOLOGIC RESPONSE OF ALLERGIC CHILDREN TO TOXOID

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IN THIS communication the immunologic response of allergic children to injections of antigen is compared with that of nonallergic children. The results of intracutaneous immunization to tetanus by the use of tetanus toxoid and to tetanus and diphtheria by the use of the combined toxoids are also here reported.

The obvious advantages of an active, as contrasted with a passive, immunity, especially when the latter necessitates the use of foreign serum, are well known. These are even more marked when we consider the transient nature of passive immunity (Figs. 1 and 2).

EXPERIMENTAL DATA

Material Used.—The material used in this study was the alum-precipitated combined diphtheria and tetanus toxoids and alum-precipitated tetanus toxoid. The diphtheria part of the mixture contained 15 to 20 Lf units per cubic centimeter. The tetanus toxoid contained 30,000 to 35,000 minimum lethal doses of tetanus toxin per cubic centimeter. The combined toxoids were standardized so that, when nine guinea pigs weighing 350 Gm. were each given one dose of 1 c.c., the blood serum of each six weeks later contained 2 units of diphtheria and tetanus antitoxin per cubic centimeter.

In the preparation of tetanus toxin, a broth made from fresh veal containing 1 per cent Berna peptone and 0.5 per cent sodium chloride was used. Before the inoculation dextrose to 1 per cent was added. After the inoculation the solution was incubated for fourteen days and then filtered through Berkefeld filters. Two-tenths per cent formalin was added, and the solutions were incubated for another fourteen days in order to detoxify them. Then the usual tests for potency, sterility, and detoxification were made.

In the preparation of alum-precipitated tetanus toxoid, alum potassium sulfate was added to the optimum point of precipitation which,

From the Otho S. A. Sprague Memorial Institute and the Children's Memorial Hospital, Chicago.

Eli Lilly & Co. supplied the tetanus and diphtheria toxoids. The tetanus toxin for titrations of serum was supplied by the National Institute of Health and Eli Lilly & Co.

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according to the United States Government requirement, must never exceed 20 mg. of alum per dose.

Subcutaneous Dosage.—Doses of 1.0 c.c. and 0.5 c.c. of the combined toxoids were used. When the alum-precipitated tetanus toxoid alone was employed, the dose was 0.5 c.c. For the primary immunization all children received two injections at intervals varying from one to three or more months. In a few instances, intervals of six months or more were allowed between the first and second injections. Stimulating or subsequent injections of either the tetanus toxoid or the combined diphtheria and tetanus toxoid have been given to some of the group six, twelve, or more months after primary immunization. The subcutaneous injections always were given in the deltoid or triceps regions of the arm. Alternate arms were used for the injections.

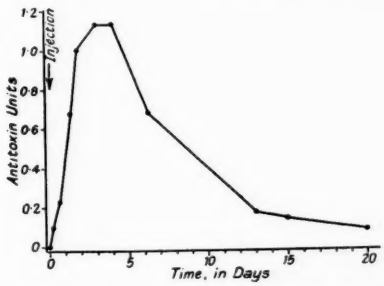

Fig. 1.—Rise and fall of antitoxin in blood of human subject after subcutaneous injection of antitoxic serum. (After Smith, from Topley and Wilson.)

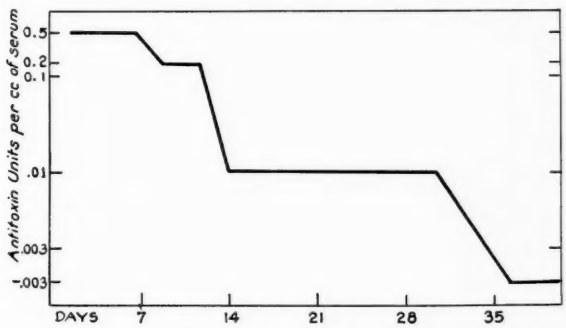

Fig. 2.—Average blood antitoxin level of sixty-four children after 1,500 U. of tetanus antitoxin.

Intracutaneous Dosage.—Doses of 0.05 c.c. and 0.1 c.c. were given when the tetanus alum-precipitated toxoid alone was used. One-tenth cubic centimeter was given when the combined alum-precipitated tetanus and diphtheria toxoid was used. The interval between the primary inoculations varied from one to three months. The interval between the primary inoculations and the stimulating doses varied from six to twelve months.

Determination of Tetanus Antitoxin Titers.—Titration of tetanus antitoxin consists in measuring serum of unknown antitoxin content against the known L+ dose of tetanus toxin. The L+ dose is that amount which, when injected with 0.1 unit of tetanus antitoxin, will just kill a 350 Gm. guinea pig on the fourth day.

Dried standard tetanus toxin was supplied by the National Institute of Health. It was diluted with physiologic saline solution so that 1 L+ dose was contained in 2 c.c. One cubic centimeter portions of varying dilutions of serum were mixed with the 2 c.c. of toxin dilution containing the L+ dose, and the mixtures were incubated at room temperature for thirty minutes. Two-tenths cubic centimeter portions of these mixtures ($\frac{1}{15}$ the amount injected when guinea pigs were used in the standard test) were injected intravenously in white mice weighing between 15 and 18 Gm. The mice were then observed for four days, and their symptoms were recorded as follows: S, Symptoms were very slight; there were beginning contraction and rigidity of the muscles of the trunk felt on grasping the animal gently, slight curvature of the trunk to one side, and stiffness of the extremities. SS, Symptoms were more definite; paralysis of the extremities was apparent; lateral deflection of the trunk and rigidity of the muscles were increased; there was slight intermittent tonic spasm on touching. SSS, Symptoms were very marked; there was extreme paralysis of the legs; the head could be drawn back, but the animal was able to rise when placed on side; tonic spasm was pronounced. SSSS, The whole body was rigid; the head was thrown back; the animal was unable to rise to its feet when placed on its side; tonic spasms occurred.

For example, when 0.2 c.c. of a mixture containing 1 c.c. of a 1:10 dilution of serum and 2 c.c. of toxin (1 L+ dose) permitted a mouse of the specified weight to live just four days, the serum contained 1.0 unit of tetanus antitoxin.

Determination of Diphtheria Antitoxin Titers.—Diphtheria antitoxin titers were determined by the intracutaneous test in rabbits, using a modification of the test reported by Fraser.[2] Two-tenths cubic centimeter doses which contained 0.001 unit of antitoxin and 0.001 L+ dose of toxin were injected. Three control tests with 0.8, 1.0 and 1.2 units were made for each rabbit, and the readings were taken after forty-eight and seventy-two hours. The tests were spaced at intervals of 0.002, 0.01, 0.1, 1.0, 2.0, 5.0, and 10.0 units. A reading of 0.1 unit indicated that the titer was more than 0.1 and less than 1.0 unit.

RESULTS

In this study seventy-seven allergic patients ranging in age from 3 months to 12 years received initial immunizing doses and stimulating doses subcutaneously. Another group of seventy-seven nonallergic patients received immunizing and stimulating doses in the same manner.

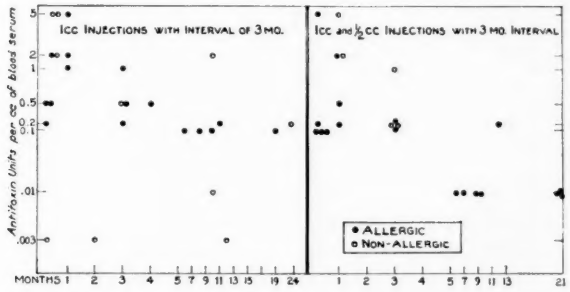
1cc INJECTIONS WITH INTERVAL OF 3 MO.
1cc and ½cc INJECTIONS WITH 3 MO. INTERVAL
Antitoxin Units per cc. of blood serum
ALLERGIC
NON-ALLERGIC
MONTHS

Fig. 3.—Tetanus antitoxin titers after two injections of combined alum toxoid.

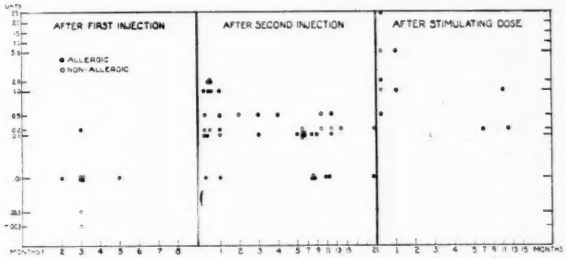

Fig. 4.—Tetanus antitoxin titers after 0.5 c.c. injections of combined alum toxoid.

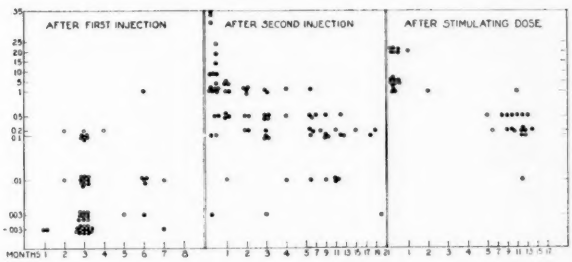

Fig. 5.—Tetanus antitoxin titers after 1 c.c. injections of combined alum toxoid.

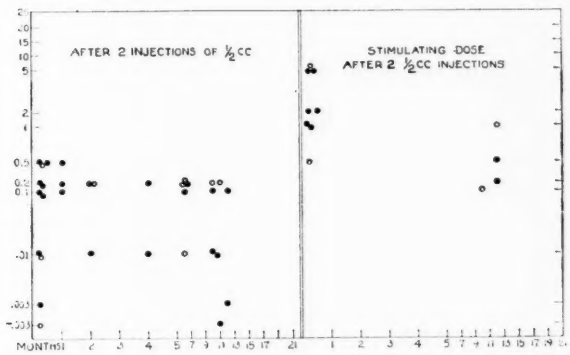

Fig. 6.—Tetanus antitoxin titers after injections of tetanus alum toxoid.

The resulting titers of both tetanus and diphtheria antitoxin produced are shown in Figs. 3-9.

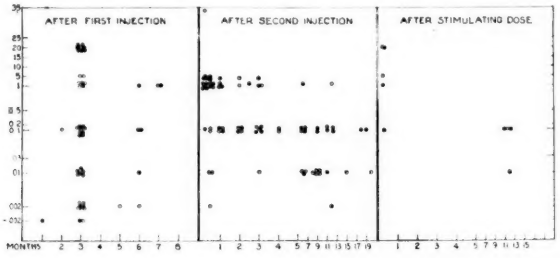

Fig. 7.—Diphtheria antitoxin titers after 1 c.c. injections of combined alum toxoid.

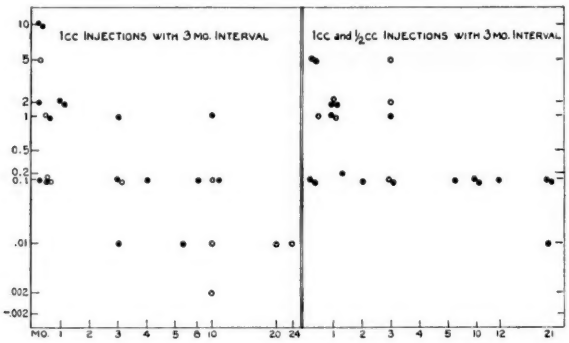

Fig. 8.—Diphtheria antitoxin titers after two injections of combined alum toxoid.

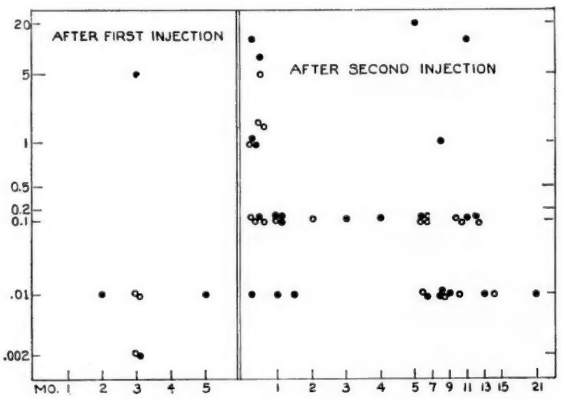

Fig. 9.—Diphtheria antitoxin titers after 0.5 c.c. injections of combined alum toxoid.

We have employed a statistical method which will remove both the age and sex factors. This is necessary when considering a group of allergic children in which the age and sex factors are pronounced, as evidenced by the preponderance of males manifesting allergic disease in the first decade of life. The only method adaptable to such a

determination is that known as "Student's" method of paired comparisons. In this method the antitoxin producing ability of an allergic child is compared with that of a nonallergic child of the same age and sex. When this method is employed, the results show that there is no difference in the antitoxin producing ability of the allergic as compared with that of the nonallergic child. Moreover, the variation among the allergic children is comparable to that found in the nonallergic group.

The interval between the injections seems to play an important role in the resulting titers. Jones[3] has shown that an increased interval between the injections results in an increased titer of diphtheria antitoxin. This also seems to hold true in the case of tetanus immunization.

INTRACUTANEOUS IMMUNIZATION

In the study of the efficacy of the intracutaneous method of immunization against tetanus and diphtheria, thirty-two patients ranging in age from 4 to 11 years received their immunizing doses by the intracutaneous method. The results of this method are shown in Figs. 10 and 11.

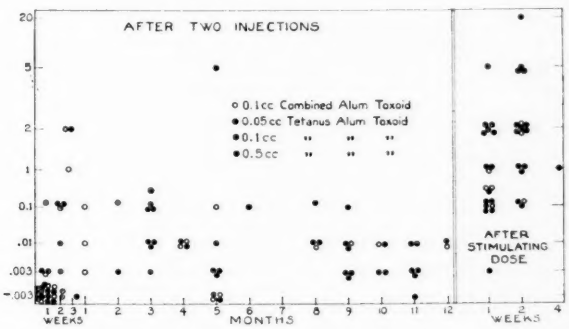

Fig. 10.—Tetanus antitoxin titers after intracutaneous injections.

By the intracutaneous method employing 0.05 c.c. of alum-precipitated tetanus toxoid, no patient developed 0.003 unit of tetanus antitoxin following the first injection. After the second injection, 50 per cent developed 0.01 to 0.1 unit of tetanus antitoxin. When 0.1 c.c. of alum-precipitated tetanus toxoid was used, two of eleven patients developed 0.003 unit of antitoxin. After the second injection of 0.1 c.c. only one patient failed to develop 0.003 unit of antitoxin. The others had titers ranging from 0.01 to 0.5 unit. When 0.1 c.c. of the combined alum-precipitated toxoids was used, no patient developed 0.003 unit of antitoxin after the first injection. All patients except one developed 0.01 to 0.1 unit following the second injection. After the second injection of 0.1 c.c. of the combined alum-precipitated toxoids, one patient did not develop 0.003 unit of tetanus antitoxin. In this patient there was also a failure of diphtheria antitoxin response. We were unable

to give this patient a stimulating dose of toxoid to determine whether there would have been a response. We[4] have observed a similar failure to produce antitoxins with two subcutaneous injections of toxoid.

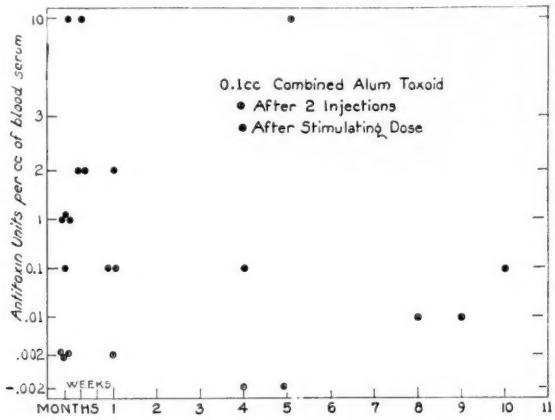

Fig. 11.—Diphtheria antitoxin titers after intracutaneous injections.

These basic titers seem fairly stable for several months following the second immunizing injection.

RESPONSE TO STIMULATING INJECTION

When either 0.05 c.c. or 0.1 c.c. of alum-precipitated tetanus toxoid was used as a stimulating dose six to twelve months after the primary immunization by two intracutaneous injections, there was an immediate, rapid, high response with tetanus antitoxin production within two weeks (Fig. 10). Only one titer was 0.1 unit; two titers, 0.2 unit; one, 0.5 unit; three, 1.0 unit; five, 2.0 units; one, 5.0 units; and one, over 20.0 units.

When the stimulating dose of 0.5 c.c. was administered subcutaneously to patients immunized with two intracutaneous injections, there was the same rapid response of antitoxin production of 2.0 to 5.0 units.

When the tetanus antitoxin titers following intracutaneous immunization were compared with those following subcutaneous immunization it was found that, in the latter instance, the tetanus antitoxin titers were higher. However, from our present knowledge it would seem that the intracutaneous method gives a sufficient response for protection. (Compare Figs. 3-6 with Fig. 10).

In a group of eight children given 0.1 c.c. of combined alum-precipitated tetanus and diphtheria toxoids, only one developed less than 0.002 unit of diphtheria antitoxin following the first injection. Two others had 0.002 unit. The others developed 0.1 unit to 20 units. The one patient with less than 0.002 unit was the child who developed less than 0.003 unit of tetanus antitoxin. There was a rapid response

when a stimulating injection was given to this group. Within two weeks the titers were from 1.0 to 10.0 units (Fig. 11).

Two intracutaneous injections of alum-precipitated toxoid, either tetanus or the combined tetanus and diphtheria toxoids, produced an antitoxin response to diphtheria or tetanus in all children except one. This response may not always be adequate to afford protection against tetanus or diphtheria, but it must be remembered that, according to our present knowledge, the first two injections should be considered as only a basic immunization and that a stimulating injection is recommended when possible infection with tetanus or diphtheria occurs. In all of the children to whom we have given stimulating doses of either toxoid, there has been a significant production of antitoxin when the titers produced were compared with the blood antitoxin level following the administration of antitoxic serum.

Tuft[5] reported on immunization against typhoid fever. He compared the intracutaneous method with the intramuscular and subcutaneous methods and concluded that the immunologic response occurring after intradermal injections of small doses of triple typhoid vaccine was equally as good as that produced by larger doses given by the subcutaneous or intramuscular route.

Kern, Crump, and Cope[1] compared the intracutaneous with the subcutaneous method of immunizing against diphtheria by the use of alum-precipitated toxoid. As a result of their study they concluded: (1) A single intracutaneous injection of alum-precipitated toxoid is as successful as a subcutaneous injection in producing active immunity against diphtheria, and with a dose only one-tenth of that usually employed in the subcutaneous method. (2) Intracutaneous diphtheria immunization is distinctly less likely to produce unfavorable reactions, local or general, than is the subcutaneous method. (3) Intracutaneous diphtheria immunization with alum-precipitated toxoid, therefore, should be the method of choice, especially in allergic subjects. These conclusions were based upon the use of the Schick test which may have been a factor in the antitoxin production.

Kohler and Heilmann[6] showed that rabbit serum which was injected intracutaneously always produced stronger cutaneous hypersensitivity in children than did that which was injected intravenously.

Haxthausen[7] made a similar study employing horse serum as the antigen. He compared the cutaneous reactions elicited fourteen days after injections of 1 c.c. of 10 per cent horse serum intraveneously and 0.1 c.c. of undiluted horse serum intracutaneously and immunization with ten 0.1 c.c. portions of 10 per cent horse serum injected intracutaneously. He demonstrated conclusively that the fixed antibody production was greater when primary injections were given by the intracutaneous route. However, comparative blood serum precipitin titers were not made.

REACTIONS

The local reactions following the subcutaneous injection of combined diphtheria and tetanus toxoids are not different from those following the use of alum-precipitated diphtheria toxoid alone. There was some pain at the site of the injections which persisted for a few minutes followed by the development of a nodule varying in size according to the amount injected. This nodule persisted for several weeks and slowly decreased in size. A similar condition followed the intracutaneous injection of toxoid. Immediately after the injection an area of erythema developed about the wheal produced by the injection. Following this, there appeared a small red papule, 3 to 5 mm. in diameter, which persisted from two to nine weeks after the injection.

The 154 patients included in the group who took their injections subcutaneously received a total of 462 injections without any untoward reactions, either local or general. Moreover, in the seventy-seven allergic patients who received a total of 231 injections of toxoid, we have seen no reactions which could be considered either anaphylactic or allergic in nature, despite the fact that most of these patients were also receiving injections of pollen extract or epidermal extract concurrently.

The immunizing procedure produced no exacerbation of any of the patients' allergic symptoms, nor did it cause any recurrence of previous allergic disease.

These same observations hold for the thirty-two patients who received a total of ninety-five intracutaneous injections of toxoid.

Careful inquiry revealed that none of the staff pediatricians at the Children's Memorial Hospital had ever seen any anaphylactic symptoms following the administration of diphtheria toxoid to children.

It will be noted from the following records that the anaphylactic reactions reported in the literature occurred in adults.

The appearance of anaphylactic reactions following the administration of toxoids has been reported by Whittingham,[8] who observed two occurrences. One patient who had received two injections of tetanus antitoxin eleven years prior to the toxoid, developed anaphylaxis with the usual cutaneous manifestations following his second injection of 1 c.c. of toxoid forty-six days after the first injection. The other patient had never received any foreign sera or toxoids, nor did he give a history of any allergic disease. The reaction in this patient was induced by the injection of tetanus toxoid forty-six days after the first. This was similar to that of the first patient. In both, it was demonstrated that the reactions were due to sensitivity to Witte peptone. In a group of 61,042 patients who had two 1 c.c. injections at six-week intervals, Whittingham found two patients who had acute anaphylactic reactions and twelve patients who developed slight constitutional symptoms, such as headache, joint pains, and urticaria.

Parish and Oakley[9] reported the occurrence of a general anaphylactic reaction in an adult woman with no allergic disease who had received in the previous years injections of staphylococcus toxoid and diphtheria prophylactic without showing any allergic response. In this patient the reaction was shown to be due to Witte peptone.

Cooke, Hampton, Sherman, and Stull[10] reported a case of generalized urticaria following the second injection of tetanus toxoid to an asthmatic patient. The patient gave positive skin tests to Berna and Witte peptone.

Cunningham[11] reported the occurrence of anaphylaxis produced by the second injection of toxoid in an adult woman. The patient had had serum sickness eight years previously. In this case the reaction was due also to Witte peptone, as shown by the cutaneous test.

One of us has observed two instances of urticaria following injection of tetanus toxoid in patients not included in this group.

Reactions due to Witte peptone contained in Schick test material have been observed by Pilot,[12] Parish,[13] and Bousfield.[14]

At present, a study of the incidence of development of sensitivity to Berna peptone is being made on this group of children.

CONCLUSIONS

1. Combined immunization against tetanus and diphtheria for infants and young children is possible, as evidenced by the antitoxin content of the blood serum.

2. A stimulating dose of tetanus toxoid given to an immunized child causes a rapid and high response of antitoxin.

3. It apparently is safe to administer toxoids to allergic children. Sensitization to toxoid was not found in 109 allergic children nor in a similar control group of nonallergic children. This is in contrast to the frequent sensitization to both horse and bovine antitoxic sera.

4. The intracutaneous method has the advantage of producing less local reaction than the subcutaneous method while producing antitoxic levels higher than that produced by antitoxic sera and lasting much longer.

5. Active immunization against tetanus or diphtheria in allergic children has no apparent effect on the allergic manifestation.

6. Concurrent allergic therapy has no effect on the antitoxin levels produced.

7. There is no difference in the antitoxin-producing ability of allergic as compared with nonallergic children.

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(See discussion on page 135.)

ESTROGENIC HORMONE DETERMINATIONS IN PREMENSTRUAL ASTHMA

G. L. Waldbott, M.D., and L. J. Bailey, M.D.
Detroit, Mich.

IN A recent survey on 125 consecutive cases of allergic women by Stocker,[1] twenty-two, or 17.6 per cent, stated that their symptoms were aggravated before and during menstruation. This has been the finding of many clinicians. The asthmatic symptoms usually improve immediately upon the onset of the menstrual flow. Among the explanations for this phenomenon are (1) an unusual state of irritability and its effect on the vegetative nervous system, (2) the possibility that a patient might be sensitive to progestin and theelin* which are present in the blood stream before the menses; (3) the existence of an increased amount of, or a lack of, certain glandular substances in the blood premenstrually. The hormone which first comes to mind in this connection is theelin.

Drips and Brunsting[2] reported a case of urticaria associated with menstrual irregularity in which estrogens were absent from the urine and estrogenic therapy proved to be beneficial. In a case of dermatitis associated with dysmenorrhea, described by Urbach,[3] estrogens could not be demonstrated in the urine. This patient, however, obtained no benefit from estrogenic therapy. Interruption of the cyclic ovarian activity first by pregnancy, and later by x-ray treatment, resulted in improvement of the dermatitis.

These experiences bring up the question whether or not a change of the physiologic estrogen content of the blood takes place in allergic persons who suffer aggravation of their symptoms premenstrually. We attempted to study this matter by determination of estrogen in the blood premenstrually. Blood studies rather than urine determinations were chosen because of the greater ease of performance of the former. Normally, estrogen is elaborated during the menstrual cycle, first by the follicle and later by the corpus luteum. Estrogens are constantly present in the blood stream during the intermenstruum, reaching a peak in the week preceding the onset of the flow, at which time they can be shown by the Frank and Goldberger test.[4] The factors which determine

Read at the Annual Meeting of the American Association for the Study of Allergy, Cleveland, Ohio, June 2, 1941.

*For several years we have systematically skin tested all women patients for theelin and antuitrin S. Occasionally we noted a more or less marked positive reaction to one of these products, but we were unable to detect any correlation with clinical symptoms.

the blood estrogen levels are ovarian activity, the rate of destruction of estrogens in the liver, and excretion in the urine which varies within not too widely spaced limits.

METHOD

Forty cubic centimeters of blood were drawn from the median basilic vein between the second and fifth day prior to the menstruation. The blood was added to 40 Gm. of anhydrous sodium sulfate. The resulting powder was extracted with ether, the extracted lipoids being taken up in 1 c.c. of sesame oil. The extract was then injected into a castrated white mouse on which vaginal smears were made. A positive smear indicating the presence of a normal quantity of theelin is demonstrated in Fig. 1. A negative smear is seen in Fig. 2. Our animals were primed every three weeks by oral administration of estrogenic substance in order to ascertain their responsiveness. With this technique Mazer[5] obtained 94 per cent positive reactions on normal women, and Sears and his co-workers,[6] 92 per cent.

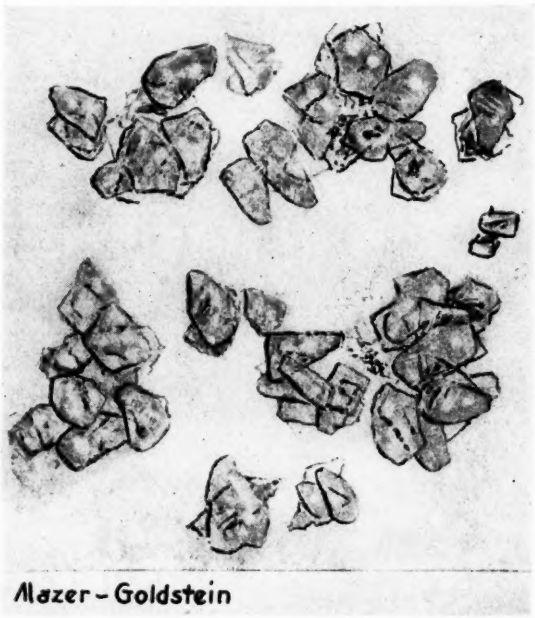

Fig. 1.—Positive estrous smear made up solely of cornified epithelium cells. (Reproduced by permission from Mazer, C., and Goldstein, L.: Clinical Endocrinology of the Female, Philadelphia, 1933, W. B. Saunders Co.)

The determinations were made on fifty-six allergic patients who gave a history of premenstrual aggravation and on twenty-three without such a history. In most of these patients the major diagnosis was bronchial asthma. The first group of fifty-six included eight with perennial vasomotor rhinitis, five with urticaria, three with eczema, two with migraine, and one with chronic allergic conjunctivitis. In the second

group of twenty-three there were nineteen asthmatic patients and four cases of chronic vasomotor rhinitis. While our patients were not subjected to special endocrinological studies, it was evident that most cases were of normal appearance. In Group I there were three who were considerably overweight and conformed to the usual concept of hypopituitarism. Three others manifested gross evidence of hypothyroidism, and one showed hyperthyroidism. Concerning the menstrual history, only five patients were irregular, three of whom menstruated every two to three weeks. Another menstruated about every three to four months when she had asthma but was regular when she was free from attacks. The number of patients complaining of more or less pronounced dysmenorrhea (ten of fifty-six) is perhaps not more striking than that in any other group of women. Also in regard to the age of the first menses (13 years on the average) the group as a whole showed nothing unusual.

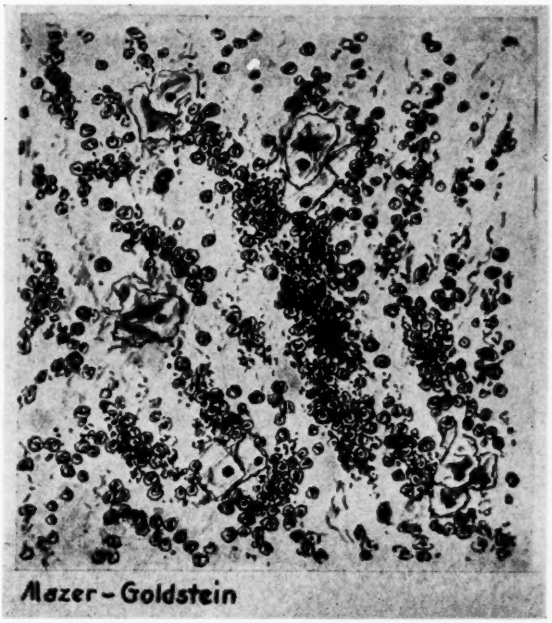

Fig. 2.—Negative (anestrous) smear made up of leucocytes and a few small, round epithelium cells. (Reproduced by permission from Mazer, C., and Goldstein, L.: Clinical Endocrinology of the Female, Philadelphia, 1933, W. B. Saunders Co.)

RESULTS

Of the fifty-six patients who gave a history of having premenstrual aggravation of symptoms (Table I), thirty-five exhibited negative, five a questionably positive, and sixteen positive tests. The twenty-three patients without such a history showed fifteen negative, one questionable, and seven positive tests. Blood was also taken on forty-two nonallergic women who had a normal menstrual history. Then control tests were run under the same experimental conditions. Thirty-seven of the

forty-two (88.1 per cent) were positive; three, questionably positive; and two, negative. Thus, in the two groups of seventy-nine allergic patients, 63.3 per cent gave a negative test as contrasted with only 4.7 per cent recorded for the nonallergic controls.

TABLE I

RESULTS OF BLOOD ESTROGEN DETERMINATIONS ON THREE GROUPS OF PATIENTS (THOSE WITH AND THOSE WITHOUT PREMENSTRUAL AGGRAVATION, AND THE NONALLERGIC CONTROLS)

DIAGNOSIS*	NO. OF CASES	WITH PREMENSTRUAL AGGRAVATION			NO. OF CASES	WITHOUT PREMENSTRUAL AGGRAVATION		
		−	+	±		−	+	±
Asthma	37	23	10	4	19	13	5	1
Vasomotor rhinitis	8	5	2	1	4	2	2	
Urticaria	5	2	3					
Eczema	3	2	1					
Migraine	2	2						
Conjunctivitis	1	1						
Total	56	35	16	5	23	15	7	1
		62.5%	28.6%	8.9%		65.2%	30.4%	4.3%

*In patients afflicted with several allergic diseases, only the dominant one is named. Nonallergic controls (42 cases): 37, +; 2, −; 3, ±.

Two factors which we believed might have a possible influence on these results were the age and the presence of symptoms at the time when the specimens were taken. Some of our patients were close to the climacteric age when negative results might be anticipated. We therefore recorded separately the seventeen cases of those who were forty or more years of age. Ten of these exhibited a negative, six a positive, and one a questionably positive test. This corresponded so well with the total group that we felt exclusion of the older patients was not indicated. A record was kept of the presence or absence of symptoms when blood was taken. Among those exhibiting allergic symptoms at that time, thirteen of fourteen showed negative tests, but also among the seventeen patients who had no symptoms, the large majority gave a negative test. We therefore could not associate the absence of estrogenic substance with allergic manifestations at the time when the blood was drawn.

In order to determine how constant our results would be, serial tests were made (between three and six determinations) on eight patients who had a negative test. The results were in line with those recorded above, but otherwise no conclusions could be drawn.

TREATMENT

Twenty-one unselected women with asthma[6] and vasomotor rhinitis[5] were given large doses (50,000 I.U.) of theelin* from one to six times

*We gratefully acknowledge the courtesy of Dr. E. A. Sharp of Parke-Davis and Company for placing this material at our disposal.

TABLE II

RESULTS OF ESTROGENIC THERAPY IN EIGHT PATIENTS WITH ALLERGIC SYMPTOMS ONLY AT THE TIME OF MENSTRUATION

NAME	AGE (YEARS)	SKIN REACTIVITY	TREATED BEFORE BY	IMPROVEMENT	MEDICATION	WHEN GIVEN†	IMPROVEMENT		REMARKS
							TEMPORARY	PERMANENT	
Mrs. M. W.	48	Little	Vaccine, pollen, diet	None	Th‡	3-8 through month	Promptly after each dose	Complete recovery	Relief of joint pain; more profuse flow
					L‡	6	None	None	
Miss M. M.	20	Little	Pollen, fungi, vaccine	None	Th	1-2	Prompt control	Considerably relieved	Recurrence if discontinued
Mrs. W. C. P.	34	Very good	Grass, ragweed, dust	Complete*	Th	2-5	None	No	
					L	3-5	Immediate	Complete control after 5 months	
Mrs. A. H.	43	Little	Diet, vaccine, fungi, histamine	Complete	Th	2-8	Occasionally improved	None	No effect on coexisting eczema
					L	3-5	None	None	
Mrs. S. H.	28	Little	Diet, vaccine	None	Th	1-3	None	None	Occasionally aggravated
					L	2-6	Definite improvement	None	
Mrs. L. M.	25	Good	Diet, pollen, fungi	Complete	Th	3-5	None	None	
					L	1-3	Relief at 3 successive menses	Better	
Mrs. F. G.	31	Very good	Ragweed preseasonally	Complete	Th	3	None	None	
					L	3	Definite		
Mrs. T. A. (Urticaria)		None	Histamine, diet, KCl, theelin	None	L	3	Immediate relief	Cured	Strong reaction (3+) to lipolutin

*"Complete" indicates control of symptoms except before menses.
†Given daily or every second day within two weeks before menses.
‡Th, 50,000 units of theelin. L, 1 mg. of lipolutin.

at one- to three-day intervals irrespective of the date of their menstruation. During the time of this treatment they were seen two to three times a week. The patients' subjective impressions as well as objective physical signs were recorded. There was no effect on the allergic condition whatsoever. We then substituted lipolutin,* P.D. in doses of 1 mg. in nine cases. This was administered in doses of 1 mg. at the same time intervals. No improvement from any of these injections was observed.

Another group consisting of fourteen patients with perennial asthma, vasomotor rhinitis, and definite aggravation of their symptoms premenstrually was selected. The latter factor, however, was considered to be of minor importance, and other causative agents were known to be active. Between two and eight doses of theelin and lipolutin were given to these women within fourteen days before the expected onset of the menses. Since other therapeutic measures were simultaneously employed with the estrogenic injections, it is difficult to draw conclusions as to their value. In three cases we felt that theelin was helpful, at least temporarily.

In Table II data are charted on eight cases in which allergic symptoms were present only before the menses. Seven had asthma, and one, urticaria. They had been treated before for such factors as pollen, dust, and food sensitivity. At the time when the estrogenic injections were given they had been completely well except for the cyclic occurrence of symptoms at the time of the menses. No other treatment was given at the time when estrogenic therapy was administered except for occasional symptomatic medication such as ephedrine and epinephrine. The estrogens were administered in the same manner as in the previous group, namely, between one and eight injections of 50,000 units of theelin or 1 mg. of lipolutin within two weeks premenstrually. Theelin was found to be of value in the control of symptoms in two patients (Cases 1 and 2), and lipolutin in three (Cases 3, 7, and 8).

SUMMARY

1. Blood estrogen determinations were done on seventy-nine allergic patients by the method of Frank and Goldberger. A total of 63.3 per cent of negative tests, indicating a relative estrogen deficiency, was obtained. This compared with 4.7 per cent in a control group of forty-two nonallergic women.

2. No marked difference in the results was noted in patients who had an aggravation of the allergic symptoms at the time of sampling.

3. Administrations of large doses of theelin and lipolutin premenstrually were of benefit only to a limited number of patients who had symptoms exclusively before and during the menstruation.

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10 PETERBORO.

DISCUSSION

DR. RALPH H. SPANGLER, Philadelphia.—I am not using the method that Dr. Waldbott has described; however, I am struck by the fact that there is a wonderfully variable factor in connection with the production of estrogenic hormone substance, and also a decidedly variable factor, as we all know, in allergy.

Dr. Fluhmann, of California, in his book on menstrual conditions, points out—and I believe well verifies—the fact that the peak of estrogenic hormone production is at the time of ovulation and not at the time of menstruation.

I believe Dr. Waldbott stated that the amount of estrogenic hormone increased at the last day of menstruation very decidedly, that is, the blood estrogenic hormone.

While I have not treated a patient such as those whom Dr. Waldbott reports, I have at the present time a patient with epilepsy, grand mal and petit mal, who has been under my care for the last four months and who seems to fit well into the group of cases Dr. Waldbott has described.

She is 30 years old and married. Petit mal started at her seventh year, and with the establishment of menstruation at 13 years she had her first grand mal attack. She is intelligent, a college graduate, and she and her mother both state she rarely has passed a menstrual cycle without a series of petit mal or a grand mal seizure which occur the day the period starts or within the subsequent week. Bromides, phenobarbital, dilantin, and thiamin chloride, the usual line of treatment, afforded little or no relief.

The patient's possible family allergic history reveals neuralgic headaches in the paternal grandfather; poison ivy dermatitis and hives (honey sensitivity) in the father, periodic migraine with vomiting in the maternal grandmother, and repeated outbreaks of hives of undetermined origin in the mother. The mother's brother, now 40 years old, is subject to periodic attacks of unconsciousness. I have never seen him.

The patient's allergic history has included bilious attacks as a child and strawberry rash, and two weeks ago I saw her with a case of ivy poisoning. This patient has been taking dilantin—had been taking it for a year before I first saw her—and I have allowed it to be continued, at the same dose, 1.5 grains t.i.d. In addition I have given an intramuscular injection of venom protein (crotalin solution) every seven days for the past four months. She has just passed her fourth men-

strual period with no epileptic manifestations occurring except two very light petit mal seizures on the day next to the last day of her most recent period.

The question arises: Is there possibly some nonspecific protein reactive effect in connection with the estrogenic treatments Dr. Waldbott has been using?

Dr. George Piness, Los Angeles.—An important factor seems to have been overlooked in this work which would have yielded more satisfactory information if hormone assays, and not merely vaginal smears, had been made.

The fact that all patients studied were asthmatic and that their symptoms were exacerbated and aggravated during the premenstrual period or the menstrual period might have significance if they did not have symptoms at other periods as well. Therefore, it is difficult to accept the theory that the presence or absence of cornified cells in vaginal secretions would indicate lack of hormone which influences bronchial asthma. Moreover, a controlled series of nonallergic patients is essential to determine whether or not the same absence of cornified cells might not be present in an equal proportion of this nonallergic group.

Dr. William P. Garver, Cleveland.—The mere fact that there is no estrogen recovered does not mean necessarily that the lack of that substance is directly influencing the asthma. Indirectly the absence of estrogen might influence asthma by removing pituitary inhibition.

We recently have had a case of a patient with a definite pituitary overactivity who developed asthma for the first time at 75 years of age. She had had vasomotor rhinitis for a number of years. We treated her with stilbestrol and estradiol, and, when vaginal bleeding became quite a problem, we treated her asthma with equal success with other pituitary inhibitory treatment such as testosterone and desoxycorticosterone. This case will be reported later.

Dr. Myron A. Weitz, Cleveland.—We all recognize with what frequency asthma and allied conditions vary with the different episodes of gonadal activity of the female, but there are certain purely clinical facts which must be remembered in this regard.

In one group are the women with seemingly normal menstrual function, but in whom the recurrence of asthmatic attacks is regularly associated with certain phases of the menstrual cycle. It is more than mere coincidence for one who has asthma daily during the premenstrual phase to have it suddenly cease with the onset of menstrual flow; in other cases for one who has continuous asthma to have severe exacerbations during certain distinct phases of the cycle, and for others to have asthma only during the menstrual phase, without symptoms at any other time.

Certain asthmas are associated with the onset of menopause and disappear completely when menopause is passed. We also know of certain cases of asthma which are inhibited during pregnancy and those which appear only during pregnancy. We also know of the influence of puberty on certain asthmas of childhood which completely disappear at this period.

I believe we are all unanimous in the acceptance of these purely clinical facts. The difficulty, however, lies in our interpretation. Only an exact understanding of the relationship between allergy and glandular physiology can permit the establishment of a basis for rationalization. Today our knowledge of glandular physiology has advanced materially compared to what it was several years ago. Nevertheless, there are still many unforged links in the chain.

The finding of low blood estrogens in the patients cited, while of possible significance, may be only part of the answer. It is difficult for me to understand why all these patients should have low estrogens. Why were the blood estrogen levels in those patients without premenstrual asthma similar to those with premenstrual symptoms? The finding of low blood estrogens presupposes good therapeutic results when these patients were given massive dosage of estrogenic hormone. Such however was not the case.

The relationships of other hormones must also be considered. It would be of some importance to know the level of urinary estrogens, because it is well known that ordinarily as blood estrogen increases, the urinary estrogen decreases; and as blood estrogen decreases, the urinary estrogen increases.

It would be well to know the effect of the anterior pituitary, the so-called "master gland," on this process. It has been suggested recently that certain persons who have asthma, also have an associated hyperinsulinism. It has been demonstrated that these persons have low blood sugars and low blood sugar tolerance curves. It has been suggested that the frequent episodes of nocturnal asthma, coming on between 3:00 and 4:00 A.M. occur because at this time the blood sugar is at its lowest. So here again is another hormonal secretion which must be considered.

The problem may be one of an allergic reaction produced by the combination of the hormone with the blood serum to produce a protein foreign to the patient. This was suggested by Urbach in a recent case report, in which he demonstrated that blood serum from a patient with dermatitis dysmenorrheica taken at a time when the dermatitis was present, when reinjected intradermally into the patient in an intermenstrual phase, produced a wheal reaction. When injected into normal females in an intermenstrual phase, it produced no reaction. Serum from a normal menstruating female when injected intradermally into the patient with dermatitis dysmenorrheica in an intermenstrual phase produced no reaction.

During the premenstruum many females experience nervousness, irritability, a tendency to cry, malaise, sensations of cold, and nausea, and some develop pretibial edema. Although psychic factors are often in the background, many authors believe that symptoms result from an endocrine disturbance. Frank termed this state premenstrual tension. He attributed the condition to an excess of estrogen in the blood, and advocated the use of magnesium sulfate in order to eliminate the hormone more rapidly. If an excess of estrogen is the cause, the administration of estrogenic substances should intensify these symptoms. This is often the case, but at times such a method of treatment brings relief. Some investigators administer progesterone and testosterone with good results. Of interest is the suggestion made that the premenstrual tension results from the generalized edema which is so often an accompaniment of this stage of the menstrual cycle. In order to offset this effect, it was suggested that patients be placed on sodium-free diets and

take 3 to 6 Gm. of ammonium chloride daily for two weeks prior to the expected period. I recently had the opportunity of utilizing this procedure in the treatment of an adult female, aged 42 years, with menstrual asthma and menstrual migraine. So far the results have been very encouraging.

DR. E. L. MACQUIDDY, Omaha.—I am interested that Dr. Waldbott did not discuss anything about the pathology of the female system. Are we to assume that all these people he had were perfectly normal?

Recently I have had a case of urticaria which persisted over eighteen months. When we made a physical examination, we found atresia of the vagina following the use of radium about five years previously. After opening up this atresia, the hives have disappeared for the past two months. Frequently D and C in some of these cases will alleviate the symptoms.

I am wondering what part pathology in this tract plays, as well as the estrogenic hormone.

DR. WALDBOTT.—I realize that in presenting these results we were sticking our necks out and I am glad that Dr. Piness brought up the question of controls. Even if he were to take 2,000 normal women, this test would be positive in about 90 per cent as is well shown in the papers by Frank and by Mazer. On the other hand, the control data available at present, for theelin assays, are much less conclusive.

Our patients had physical examinations, and we attempted to note endocrinological changes. In some we had complete endocrinological surveys, but we refrained from doing this routinely because there was no indication that these women were abnormal in this respect.

Dr. Garver, we did not attempt to explain our results. Your idea is certainly of interest to us.

Dr. Weitz brought up the question of making determinations of estrogenic substances in the urine. They are said to parallel very closely those of the blood. As to the possibility of sensitivity to estrogens, for many years we performed skin tests with theelin, antuitrin, and lipolutin on our patients. We have never found any conclusive information from doing this, with the exception of the case of urticaria, named last in Table II. This patient exhibited a very strongly positive reaction to lipolutin.

ABSTRACTS AND DISCUSSIONS OF TWO PAPERS READ AT THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR THE STUDY OF ALLERGY, CLEVELAND, OHIO, JUNE 2, 1941

Tetanus Toxoid in the Prevention of Tetanus and Serum Reactions in Allergic Persons. LEON UNGER, M.D., Chicago, Ill. (Abstract.)

Because of the demonstrated value of tetanus toxoid in the prevention of tetanus and of serum reactions a study was made on 171 unselected persons. All of these patients had one or more allergic conditions, chiefly bronchial asthma and hay fever; some also had urticaria, atopic dermatitis, or allergic rhinitis. Ages varied from 3 to 63 years; the majority were in the second and third decades. Two injections each of 0.5 c.c. of alum-precipitated tetanus toxoid were given subcutaneously in alternating arms, at intervals of approximately eight weeks.

There were no systemic reactions. Local reactions occurred in twenty-three patients; three were severe. Eight patients received combined diphtheria-tetanus toxoid, with one local reaction. Twenty-eight patients had previously received tetanus or diphtheria antitoxin, with serum sickness in eleven; only one of these had a local reaction from tetanus toxoid. In twenty-three persons with proved allergy to horse dander there were three local reactions. In seventeen other persons with moderate to strong cutaneous or intracutaneous reactions to horse dander, without proof of clinical allergy, two local reactions occurred.

It is concluded that immunization is especially indicated in those who have had antitoxin previously and in those allergic to horse dander. In these groups local reactions were not more frequent. Toxoid should also be given to all allergic persons as well as to all children and to those especially predisposed to injury.

DISCUSSION ON "THE IMMUNOLOGIC RESPONSE OF ALLERGIC CHILDREN TO TOXOID" BY DR. TOWNSEND B. FRIEDMAN, DR. JOHN A. BIGLER, AND MARIE A. WERNER;* AND "TETANUS TOXOID IN THE PREVENTION OF TETANUS AND SERUM REACTIONS IN ALLERGIC PERSONS" BY DR. UNGER

DR. M. MURRAY PESHKIN, New York City.—My discussion will be confined to the tetanus antitoxic response.

It has been assumed by some investigators that active immunization against tetanus, to be of value, must produce an antitoxin titer at least equal to that present in the blood following passive immunization with a prophylactic dose of 1,500 units of tetanus antitoxin. This arbitrary minimal or critical protective value was placed at 0.1 unit of antitoxin per cubic centimeter of serum.

*See page 114 for article by Friedman, Bigler, and Werner.

It should be borne in mind that active immunization with toxoid induces a phase of allergy, bacterial allergy, and, as such, is not even remotely comparable to the mechanism of protection induced by passive immunization; moreover, there is no scientific evidence to show that 0.1 unit in an actively immunized human subject is necessary for protection against tetanus.

Ramon, Sneath, and his associates, and Jones, among others, regarded an antitoxic response to tetanus after active immunization equal to 0.01 unit (U. S. P.) as satisfactory. Dr. Friedman, I believe, also accepts this value as a protective level. Any value less than 0.01 unit, such as 0.003 unit, may be designated as an inadequate or nonprotective level.

My experience of four years with active immunization is restricted largely to the use of combined diphtheria and tetanus toxoids, alum precipitated (of a tetanus toxicity of 30,000 M.L.D. per cubic centimeter), and is based on a series of 186 allergic children between the ages of 3 and 15 years. A primary course of injections consisted of two doses of 0.5 c.c. each, administered subcutaneously. The intervals between injections ranged from one to ten months. Doses of 1 c.c. were not employed because, from an immunologic standpoint, it did not seem likely that the antitoxic response would be appreciably greater than with the 0.5 c.c. doses. This postulate is substantiated by Dr. Friedman's work.

The advantages resting with the smaller volume of toxoid injections are obvious. Sixty-five of these children were given a third or repeat dose of the combined alum toxoids from three to fifteen months after the completion of primary immunization. The total number of titrations performed was about 1,000.

With few exceptions, the patients were also given injections of pollen and other antigens during the entire period they were under observation.

Sex played no role in the incidence of the maximum initial adequate antitoxic response or in the duration of immunity in the various groups tested.

The children of preschool age acquired adequate antitoxic immunity following two doses of combined toxoids injected at intervals of one and ten months, whereas all the children of school age acquired protection only following injections given at intervals between five and ten months.

The initial maximum antitoxic response occurred in almost all cases from seven to thirty days after alum toxoid.

Of the group of 186 children who were given two doses of toxoid, 176, or 94.6 per cent, maintained adequate antitoxin levels for a period of three months after toxoid. Thereafter, the incidence of children showing an adequate antitoxin titer was 65 per cent at one year and 43 per cent at two years. The remaining ten of the 186 children showed inadequate protection, and all of these ten children received injections of combined toxoids at intervals between one and four months. Nine of these ten children were given a third dose of toxoid, and within one month all of them responded with an adequate antitoxic immunity.

When the group of children injected with two doses of toxoid at intervals between five and ten months was compared with the group injected at intervals between one and four months, it was clearly evident that a significantly greater percentage of the former group re-

sponded to the highest antitoxic values not only within three months but also one year after the second dose of toxoid. Thus, the interval of time between the administration of two doses of alum toxoid had a direct influence on the antitoxic response, qualitatively and quantitatively.

In the group of sixty-five children injected with a third dose of toxoid, it was found that the repeat injection was followed by an adequate and more durable tetanus antitoxin titer as well as a titer of higher value lasting for a much longer period of time than that which followed the second injection even though the corresponding titer obtained within one month of the third injection was the same or less.

Local reactions were harmless. The incidence of local reactions after the third injection, as compared to the corresponding reactions after the primary two injections, was about 25 per cent for each group. An elevation of temperature lasting about one day followed the third dose in 3 per cent of the children injected, as compared to 1 per cent of the children given two doses of combined toxoids.

Since only allergic children were the subjects under investigation, it is significant that no allergic reactions, such as urticaria, asthma, or other allergic symptoms, were encountered. Dr. Friedman reported similar findings. Moreover, all of the children who were tested by the scratch method with the undiluted combined alum toxoids reacted negatively to the various substances in the toxoid preparation. The tests were performed from two months to two years after the second and third doses, respectively.

In the absence of any practical test, such as the Schick test for diphtheria, any formulated plan of treatment with tetanus alum-precipitated toxoid must be depended on to induce adequate antitoxic immunity in the children treated.

Since the development of active immunity against tetanus was influenced by age and interval of time between injections and in order to insure adequate active protection against tetanus in all children treated, it appears reasonable to state from the results obtained in my studies that for primary immunization the criterion of adequate tetanus antitoxic immunity is the administration of three doses (0.5 c.c. each) of alum-precipitated toxoid at intervals of at least one month or more. However, Dr. Friedman found that two doses of 1 c.c. each given at intervals of three months resulted in adequate protection.

Until a large number of children in this country are vaccinated over a considerable period of time, and despite the demonstration of the practical value of active immunization against tetanus, nevertheless, it is recommended that at the time of injury a reinjection or stimulating dose of tetanus alum-precipitated toxoid be given.

Finally, should exposure to tetanus occur before vaccination is completed and immunity is established, in seven to thirty days, a simultaneous injection of antitetanus serum and toxoid (serovaccination) should be administered.

DR. UNGER.—Dr. Friedman's paper and mine were in agreement with the exception of two points. Ramon and Zoeller and others report that the use of combined toxoid enhances the value of each one of them. Dr. Friedman, I noticed, says it does not and that agrees with what Otten and Henneman reported. Dr. Friedman also said he had seen no particularly severe reactions in children. His age groups are younger

than mine. The youngest I had was 3, and the children ranged in years from 3 to 15. We had five rather severe local reactions in twenty-three cases; they do occur in older children.

I want to take this opportunity to urge all of you to immunize your allergic patients with available toxoids. Some allergists have been a little lax. They feel they are doing allergy and do not want to step outside the borders of it. Immunization is at least closely related to allergy.

DR. FRIEDMAN.—I want to state again that we reported facts. There was no philosophy or metaphysics about it, and no enhancing effect of one toxoid by the other was found.

As to the protective level, we purposely avoided saying anything about that. We gave tetanus antitoxin and compared the titers with what occurred following active immunization. In children, we found no reactions, local or general, to injections of toxoid.

Pollen in Oil. A Preliminary Report on a New Slowly Absorbed Medium for Use in Hay Fever Treatment. SAMUEL J. TAUB, M.D., and ELI RUBENS, M.D., Chicago, Ill. (Abstract.)

An aqueous pollen extract was prepared and then rapidly frozen. The residue was dissolved in sterile sesame oil. Intradermal tests were made on 200 ragweed-sensitive patients, and delayed positive reactions resulted in all of them. Reactions consisted of erythema and itching coming on thirty to forty-five minutes after the intradermal test. Control tests with sesame oil were negative.

The active principle is present in these oil extracts as evidenced by the local reactions produced in sensitive patients. A slower rate of absorption can be demonstrated with less likelihood of severe local and systemic reactions.

Higher concentrations of pollen extract are possible with pollen in oil, and therefore patients can be given a single large dose with no danger of constitutional reactions.

These extracts do not deteriorate at room temperature and need not be refrigerated.

DISCUSSION

DR. GRAFTON TYLER BROWN, Washington, D. C.—This idea of pollen in oil for the treatment of hay fever is an intriguing one. The principal advantage of using preparations of pollen in oil would be their slowness of absorption and, consequently, lessened likelihood of producing constitutional reactions.

Somewhat similarly, Naterman, in 1938, treated hay fever with pollen extracts emulsified in lanolin and olive oil and claimed that these emulsions were absorbed much more slowly and hence large doses could be administered with less chance of constitutional reactions. Naterman also found that fewer injections were necessary for a satisfactory course of treatment.

Certain possible disadvantages come to mind, however, in considering the use of oil as a medium for pollen extracts. One is the possibility of sensitization to sesame-seed oil, even though none has so far been reported. Among other uses, sesame-seed oil is used as a vehicle for iodine for intrabronchial use, and also for estrogenic hormones for intramuscular injection.

Dr. Taub refers to frequent reports of sensitization to peanut and poppy-seed oils. Of course, peanuts and poppy seeds and their products are more widely used than sesame seed and sesame-seed oil, but all of the seeds are rich in protein and notorious sensitizers, and I am one who believes that the oils from these seeds retain some of the allergenic activity of the mother seed.

The most severe constitutional reactions from pollen inoculations are those that occur immediately, from accidental injection of some or all of the dose into a small blood vessel, and no form of oil would prevent this type of reaction.

Another possible disadvantage is that the extracts in oil would be so slowly absorbed from the tissues as to cause overlapping of doses and summation of action in the system, thus preventing building up to a sufficiently large preseasonal dose within a reasonable period of time. This thought was suggested by the authors' mention of the reappearance of a raised red papule with itching, after a quiescent period of one week following an intradermal test with pollen in oil. Perhaps, therefore, the most logical use for these pollen extracts in oil would be in the coseasonal treatment of hay fever, where it would seem advantageous to establish a depot of pollen extract in the tissues from which relatively small amounts would be slowly liberated over a period of a week or longer.

Furthermore, in the absence of any definite whealing with pseudopods from intradermal testing, I question the potency of these extracts in oil. It also remains to be proved whether any pollen extract retains its potency as well when kept at room temperature as when stored in the refrigerator.

Traces of water cloud and spoil oil extracts. This necessitates using absolutely dry syringes and needles, which is an inconvenience as most physicians use wet sterilization of syringes.

Another argument that has been advanced against extracts in oil for injection is that unabsorbed oil may cause tumefaction or even malignant changes. This complication must be rather remote, however, considering the tremendous number of estrogenic hormones in oil that are being injected.

From an extensive experience, I believe that 50 per cent glycerosaline pollen extracts possess all of the advantages of pollen extracts in oil, with none of their disadvantages. Fifty per cent glycerine definitely slows the rate of absorption of pollen extracts and thus keeps down constitutional reactions. It also maintains the potency and sterility of the extracts; and it is only slightly painful on injection. I believe this opinion of glycerine extracts has been supported by the work of Clock, Harrison, Bernton, Aaron Brown, and a number of others who have used the 50 per cent glycerine extracting fluid.

Dr. Samuel M. Feinberg, Chicago.—I believe there is some theoretical merit in trying to achieve what Dr. Taub is trying to achieve, and that

is something that will delay absorption of an antigen. I had the idea, as far back as ten years ago, that anything that might delay absorption might decrease the number of doses used and decrease systemic reactions and might possibly increase the effectiveness of the treatment; so, at that time we began to work on preparations for delayed absorption.

Our first attempts were the very attempts Dr. Taub has spoken about, with the exception that we used materials which were dried in other ways and suspended in oils, or precipitated by various precipitants and suspended in oil. Later on we thought we might be missing something in the whole pollen, and we actually ground up the pollen and suspended that in oil. Of course, a large amount of unabsorbed material and debris was left. Then we thought we were not getting very far with that method, so we made emulsions of various kinds, soap emulsions, and water-continuous emulsions, and finally oil. Then the report of Dr. Naterman appeared, and we duplicated his work with lanolin emulsions.

Now, working with various kinds of emulsions, with the most perfect emulsions so far obtainable, we have come to the conclusions that, on the basis of experiments on absorption with passive transfer methods, there is some delay of absorption, but that delay is not sufficient with the present methods to achieve that which may be desired.

Furthermore, over several seasons we tested these various emulsions therapeutically, and, although they have enabled us to decrease the number of injections, constitutional reactions have not been entirely eliminated so far. We are still working on the problem because we feel that the concept Dr. Taub has expressed here is correct, and finally someone will achieve a more perfect type of material for delayed absorption which will be useful clinically.

I was puzzled, from the abstract, as to how this material could be dissolved in oil. I think the mystery has been cleared up now. As I understand it, the material is suspended in oil, not dissolved. There is still a question in our minds, and we have been working at it for several seasons, wondering if we can achieve this still unattainable perfection of delayed absorption without producing undesirable tissue reactions. We have had materials which produced ideally delayed absorption, in which tissue reactions were so violent, as indicated in animal experiments, that we would not dare use them in patients. Naturally we must find a happy medium between these two effects, a moderate delay and yet not so marked that there will be too much tissue reaction. Yet, when we achieve this end, if we do, I am not certain whether we shall actually be able to do more for our patients other than merely to cut down the number of doses.

DR. ARMAND E. COHEN, Louisville.—During the 1940 ragweed season, I treated, with ragweed suspended in a 16 per cent gelatin solution, six patients who previously had suffered general reactions from glycerine-saline ragweed extract. My results were similar to those of W. C. Spain, who has published a comprehensive report dealing with this subject (THE JOURNAL OF ALLERGY, May, 1941).

In order to get comparative results in dealing with these extracts, it might be well to use the pollen appearance time technique as suggested by Dr. Milton Cohen.

I believe the oily solutions are less physiologic than gelatin, and there is some danger from oil emboli. Again the oils are more likely to produce tissue sensitivity in these atopic patients.

There are instances in which general reactions might be prevented by more slowly absorbed pollen extracts. The use of a pollen extract which will make these reactions less frequent is desirable.

DR. THEODORE B. BERNSTEIN, Chicago.—Dr. Feinberg has intimated he has been interested in this subject of delayed absorption for some time. I have been associated with Dr. Feinberg for several years, and together we have encountered many of the difficulties inherent to this problem.

Some time ago we had found that dried ragweed extract was not soluble in almond or peanut oil. After reading the abstract of the paper presented by Dr. Taub, it was decided to do the work over again, and to use this time sesame oil, with the thought that perhaps this oil had physical or chemical characteristics differing from those possessed by the other oils. I should like to say first that we found a dry pollen extract was not directly soluble in oil. It made no difference whether we examined the material after a few hours or allowed it to stand around for a few days, or even a month; we still found gross evidence of a solid material on the bottom of the container. Various types of agitation did not help.

There was still the possibility that a small portion of the lyophiled material was soluble in the oil and that this part might be the reaction-producing fraction. Therefore, nitrogen analyses were made of the 3 per cent aqueous ragweed extract, the sesame oil, and the centrifuged-off sesame oil that had been added to the dried ragweed extract and allowed to stand for forty-eight hours.

(Slide) It will be seen that our 3 per cent aqueous ragweed extract contained total nitrogen of approximately 0.7 mg. per cubic centimeter, and that the protein nitrogen content of the material was 0.26 mg. per cubic centimeter. The sesame oil, before addition to the dried ragweed extract, contained approximately 0.6 mg. of total nitrogen per cubic centimeter, and the amount of protein nitrogen was so small that it was negligible. Then, after this oil had been added to the dried ragweed material, it was centrifuged off and again subjected to analysis. At that time we found a total nitrogen of 0.66 mg. per cubic centimeter and a protein nitrogen content also that was very small. It appears, therefore, that, if any of the active principles are soluble, the amount is very minute.

(Slide) We then tested fifteen hay fever patients by the scratch and intradermal methods with what was considered to be a 6 per cent ragweed sesame oil. (The dried material was taken up with sesame oil in half its original volume.) Although the 3 per cent aqueous ragweed extract gave three-plus intradermal reactions, in dilutions as high as one to ten million in all of these fifteen patients, no reactions were obtained with the ragweed-sesame oil material either by the scratch method with the 6 per cent concentration, or intradermally with dilutions of 1:10,000, 1:1,000, or 1:100. In some cases small red flares appeared, but in these patients the same type of flare appeared both in regard to size and in regard to time with the sesame oil control. Con-

sequently, we figured that the reaction was not a specific one and was due mainly to the oil itself. These reactions usually appeared after five or ten minutes.

DR. BEN Z. RAPPAPORT, Chicago.—I had hoped that Dr. Voss would be here to discuss the results that he published on the aluminum cream adsorbed pollen. We did some work of that nature about five years ago and did not publish the results because of the negligible effect as compared with ordinary treatment. Either there was no improvement over ordinary treatment or even less improvement at that time.

At the University of Illinois we treated about eighty cases with aluminum hydroxide cream and pollen—pollen adsorbed on aluminum hydroxide cream. There was no question about the severe local reactions that we could obtain with the material, although there was no way of testing with the substance intracutaneously or by scratch method, because of the nature of the product.

Despite the fact that we could get very large local swellings and delayed absorption, a delay of two or three weeks as indicated by the local appearance of the swelling and the prolonged period of local itching, the therapeutic results were no better or probably were worse than in the ordinary course of treatment.

When we speak of delayed absorption, we must be sure that there is delayed absorption. Just because one injects an oil emulsion of pollen or an aluminum-adsorbed pollen is no indication that there is a regular degree of absorption of a certain amount, for a certain period of time. After all, once you inject it into the body, there are biologic processes, enzyme actions, actual chemical reactions, and also physical reactions which may result in a rather rapid and sudden separation of the material from the "slowing agent." The separation, when it reaches a certain stage, may be rather sudden, so that one has to beware of thinking in physical terms of the material outside the body and the state of the material once injected in active, living tissue.

DR. I. S. KAHN, San Antonio.—My observation on sesame oil may not be very important in this connection, but I do see many patients with allergy who use it; probably many more than you gentlemen do, for the reason that sesame oil and meal are not in very common dietary use in the United States, but are very extensively used by the natives of Mexico and by Texas-born Mexicans. In fact, they are used by them almost daily. I have been watching for sesame sensitivity, but as yet, have never encountered a case.

DR. TAUB.—I wish to thank the discussants for their discussions and suggestions, and I repeat again that this is just a preliminary report showing that we have been able to produce reactions in which we think we have the active principle of the pollens suspended in the oil, that seems to be active on skin tests.

I do not know what technique Dr. Feinberg and Dr. Bernstein used, but in their ten years of work with this, they apparently tested but fifteen patients and obtained negative reactions. They should repeat this work using more patients and an active extract.

The active principle is present in these oil extracts as evidenced by the local reactions produced in sensitive patients. A slower rate of absorption is demonstrated with less likelihood of severe local and systemic reactions.

Higher concentrations of pollen extract are possible with pollen in oil, and therefore patients can be given a single large dose with no danger of constitutional reactions.

The aqueous pollen mixture is frozen rapidly in the carbon dioxide-carbon tetrachloride mixture and then evaporated to dryness in vacuo. After this procedure it is redissolved in sterile sesame oil with constant agitation.

A further report with results in treatment of hay fever patients will be made in the near future.

Original Articles

THE EXTRACTION OF RAGWEED POLLEN AS OBSERVED WITH THE ULTRAMICROSCOPE

ETHAN ALLAN BROWN, M.R.C.S. (LONDON), L.R.C.P. (ENGLAND), AND NORBERT BENOTTI, B.S., M.S., BOSTON, MASS.

EXTRACTS of ragweed pollen have for many years been used for the treatment of allergic conditions. It is a matter of common knowledge that the pollen grains as such do not themselves dissolve in the extracting fluid. Exactly how the pollen protein goes into solution has always, therefore, been a matter of conjecture. Some physical phenomenon does, however, take place, since, by simple analysis, the extract can be shown to contain both organic and inorganic substances.

It occurred to us that the resolution of pollen in solution might fall within the province of colloidal chemistry and that the ultramicroscope and the electron microscope might, between them, make visible a number of phenomena previously suspected but not hitherto described as having been seen. This proved to be true. The present preliminary note is concerned with the visible mechanisms of pollen extraction.

The ultramicroscope* used was of the double oil immersion type with a cardioid condenser, an oil layer lying both above the cover slip and below the slide.[1] This optical system makes it possible for the observer simultaneously to examine the pollen grains as microscopic objects and the colloid particles as ultramicroscopic.

A few grains of undefatted short ragweed pollen were dusted upon an ultramicroscope slide with a camel's-hair brush. The extracting fluids used were distilled water, physiologic saline, buffered saline,[2] and Coca's solution.[3] A few drops of the chosen extracting fluid were added to the pollen, and a cover slip was put in place. The excess fluid was removed, and the edges of the cover slip were sealed to the slide with paraffin.

It was apparent within a few minutes that all of the extracting fluids used gave similar patterns. The field appeared uniformly spread with colloid particles in Brownian movement.

With the buffered saline solutions, a phenomenon, which merits detailed description, was observed. From the pores or the grooves of the

From the Asthma Research Foundation, Boston.

*Placed at our disposal by Dr. Ernst A. Hauser, Associate Professor of Chemical Engineering, Massachusetts Institute of Technology, who has, with great courtesy, given us freely of his time and knowledge.

pollen grains, fine tenuous threads grew out extending radially. Occasionally, when several were present, they extended in the same direction as though current movements in the extracting fluid had forced them into these new positions. Along these threads, and arranged like beads on a string, were the colloid particles. After thirty minutes, the threads were no longer visible (perhaps due to resolution in the extract), and the microscope field then appeared to contain the colloid particles spread uniformly and in Brownian movement.

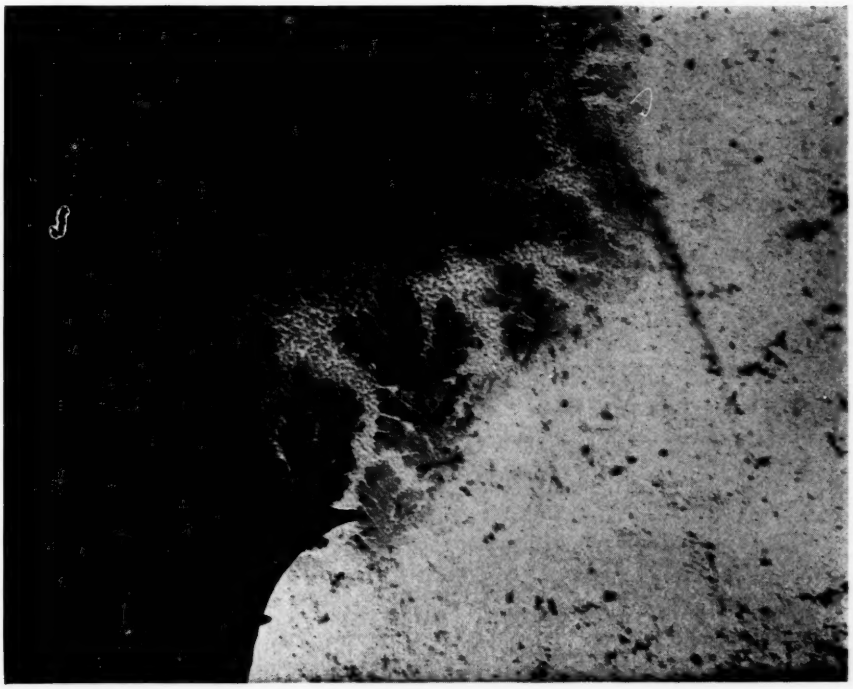

Fig. 1.—Pollen grain showing disintegration. (×48,000.)

With the physiologic saline solution, the pollen grains seem to swell before releasing their contents, which appeared as an irregular mass of spherical bodies. These slowly dispersed, and, within an hour, the field resembled that seen with all of the extracting fluids.

With distilled water and with Coca's solution, the mechanism by means of which the particles left the pollen grain was not visible, but the appearance of the field, after a suitable time had elapsed, was as described above.

Within twenty-four hours, and visible in the same extract, were a number of rods, infinitesimal in size but of the proportions of diphtheria bacilli. These were first observed in ordinary extract and were thought to be due to particles from the Seitz filter. They can be seen, however,

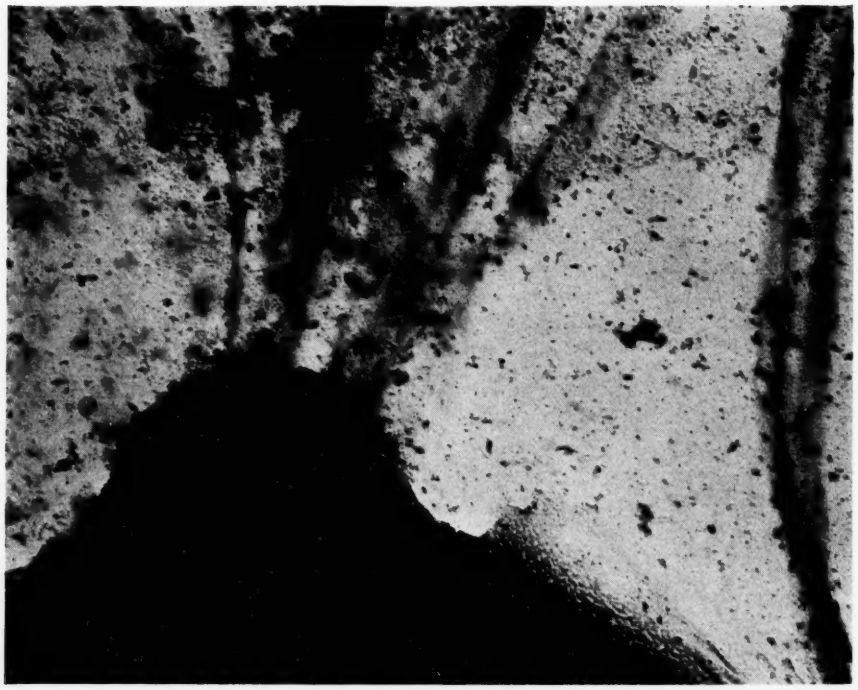

Fig. 2.—Disintegration further advanced than in Fig. 1. (×48,000.)

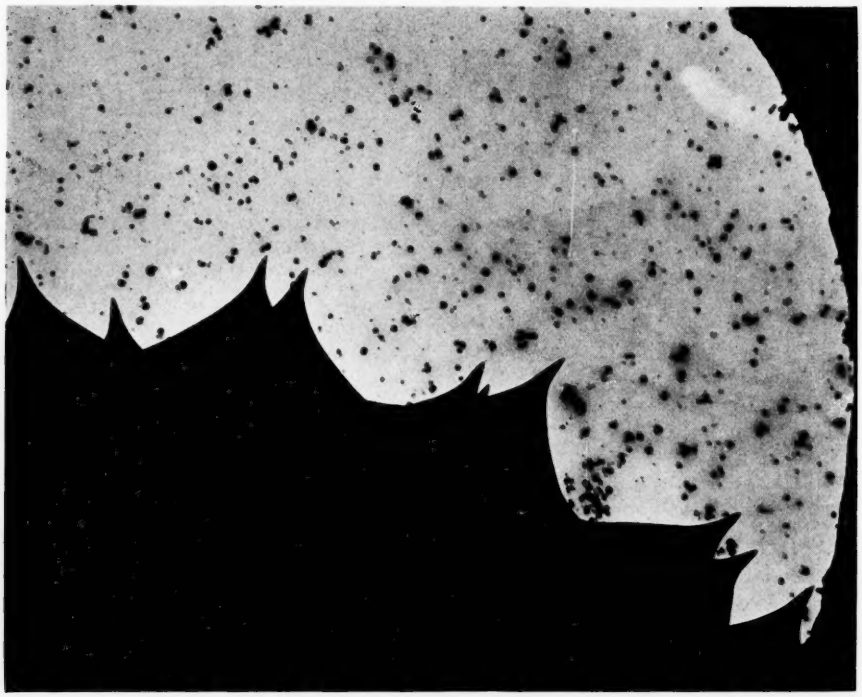

Fig. 3.—Short ragweed pollen. Pollen (black) is shown in silhouette. About one-eighth of the circumference is visible. (×48,000) $1\mu = 4.8$ cm.

in unfiltered extracts, in purified albumin, and also in the globulins obtained from the fractionation of aqueous pollen extracts. Means are now being devised for the investigation of their physical and chemical properties.

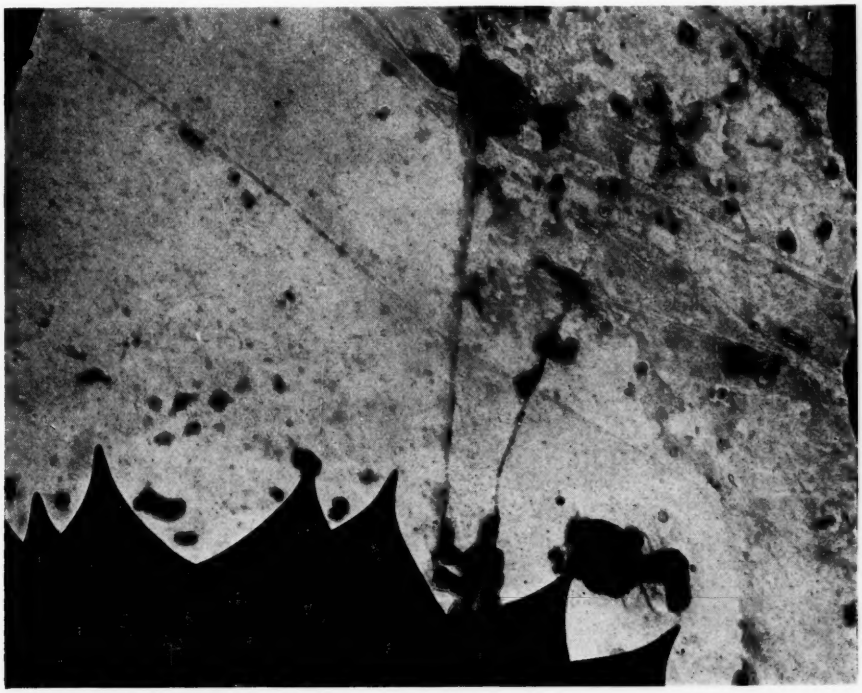

Fig. 4.—Short ragweed pollen. The edge of the pollen granule is seen as black and in silhouette. With this magnification about one-twelfth of the circumference of the pollen grain can be seen. (×48,000) 1μ = 4.8 cm.

In addition to the above observations, certain other facts regarding pollen were discovered. It was noticed that, with a slight pressure on the cover slip, the pollen grains expelled a jellylike mass of spherical bodies which slowly dispersed into the colloid particles. There were, of course, none of the threads and beads as seen in the buffered saline extraction. Physical methods, as by pressure or ball milling, appear to increase the number of colloid particles present, although the antigenicity of the solution evidently does not vary greatly.[4]

With the electron microscope,* studies can be done only on dry materials, since the microscope operates in an almost perfect vacuum. Attempts were made to photograph the threads and beads, but in the dry state they evidently disintegrate. It was possible, however, to get a photograph of ragweed pollen by electronic discharge. In Figs. 1-4, these ragweed pollen grains are shown multiplied 48,000 times. This is the equivalent of 1 foot extended to over 9 miles, or 1 square foot, to

*Placed at our disposal by the Research Laboratories of the American Cyanamid Company, at Stamford, Conn.

more than 81 square miles. One of the grains was seen discharging its contents, and Figs. 1 and 2 show the point at which the material was expelled. There are a number of lines extending from the point of expulsion. These lines do not show the sharp definition of the threads as seen under the ultramicroscope, and no colloid beads are visible.

The techniques described above are now being used for the study of antigen-antibody relationships by optical techniques.

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STUDIES ON THE IMMUNOLOGY OF RAGWEED POLLEN PROTEINS

II. Anaphylactic Experiments

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IN A previous communication[1] members of our staff were able to show that short ragweed pollen extract when subjected to a temperature of 60° C. at a pH of 4.0 gave a precipitate, which apparently was of large molecular size, and a filtrate of small molecular size. Skin tests performed with the former showed definitely smaller swellings as compared with similar tests made with untreated short ragweed pollen extract at the same total nitrogen value. The filtrate gave skin reactions which compared favorably with those given by untreated short ragweed pollen extract at an equivalent total nitrogen value. Precipitin tests with short ragweed pollen antiserum revealed that the large molecular material gave marked precipitin reactions while the small molecular material gave no reactions. The hydrogen-ion concentration of pH 4.0 was chosen because preliminary work had demonstrated that that was close to the isoelectric point of short ragweed pollen extract.

After the above report appeared Cohen and Nelson,[2] working with a ragweed extract of small molecular size, prepared according to our specifications, observed an increase in the inhibition antibody of Cooke following injection of the material into sheep. Recently Abramson[3] by delicate electrochemical methods has been able to prove that the isoelectric point of ragweed pollen extract is pH 3.9. In addition he studied the water-clear ultrafiltrate obtained from ragweed extracts and found great activity when it was used as skin test material on ragweed sensitive patients.

Stull and Sherman[4] have studied the problem of protein and nonprotein fractions of ragweed pollen. They believe that the allergenic activity is associated with the protein fractions. Benjamins and his collaborators[5] have also shown the skin reactivity of small molecular material obtained from grass pollens.

In the present study we repeated our previous work and were able to verify the previous conclusions in regard to the skin test and precipitin reactions. In addition we studied the anaphylactic reactions with the small and large molecular material. Twenty-one guinea pigs were used. Twelve of these guinea pigs were injected intraperitoneally with short

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ragweed extract, each animal receiving an amount of dilution equivalent to 0.4 mg. of total nitrogen. The shocking dose in all cases (whether the material was large, small, or original pollen extract) was uniform, i.e., 1.0 mg. of total nitrogen. Fourteen days later four of these pigs were given intracardial shocking doses of small molecular material which had been previously neutralized. These animals showed no reaction. Forty-eight hours later these animals were injected with short ragweed extract, and all reacted with violent anaphylactic shock although no death occurred. Three normal control guinea pigs were injected with a diluent containing the same salts at the same concentration and at the same pH as the small molecular solution but containing no pollen material. These pigs gave no reaction.

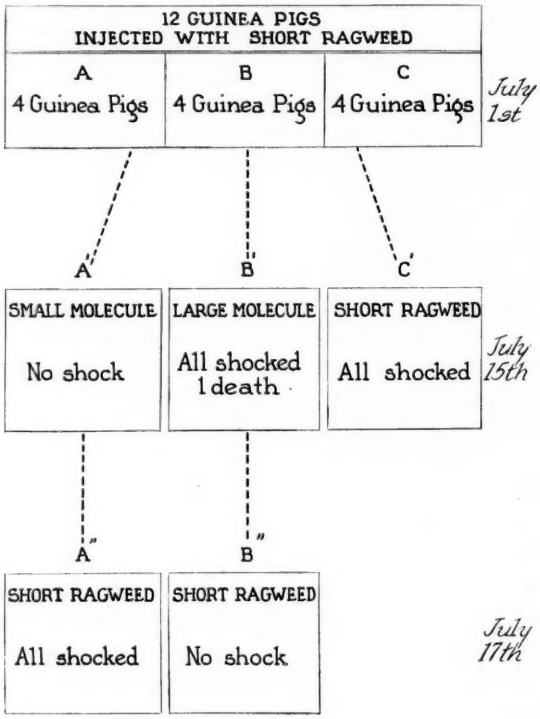

Fig. 1.

Four of the sensitized pigs were injected with short ragweed pollen extract and presented immediate severe anaphylactic shock. No death occurred. Three of the untreated control animals were injected with the same short ragweed extract and showed no symptoms.

Four of the sensitized guinea pigs were injected with an extract of the large molecular material and went into immediate severe anaphylactic shock. One of these guinea pigs died within one minute after the injection. The surviving guinea pigs were injected forty-eight hours later with short ragweed pollen extract and gave no reaction, thus

showing desensitization. Three of the untreated control guinea pigs were injected with a diluent containing the same salts at the same concentrations and at the same pH as the large molecular solution except for the absence of pollen material and gave no reaction.

DISCUSSION

The above experiments demonstrate that the small molecular material does not possess the capacity to elicit anaphylactic shock. The large molecular material shows this capacity, as does the original pollen extract. There are two possible explanations for these observed phenomena. (1) The large molecular material is responsible for the precipitin and the anaphylactic reactions. (2) The small molecule is responsible for the precipitin and anaphylactic reaction providing it is adsorbed on a large molecular aggregate acting as a "schlepper," whereas when in the pure state it is incapable of causing these reactions.

The results of the skin tests reported in our previous communication[1] indicated that the large molecular material gave definitely less severe skin reactions than the small material at an equivalent nitrogen value. An explanation previously advanced was that the large molecular material had been denatured in its preparation and thus gave less positive skin tests. This may not be the correct explanation for the diminished skin reaction, but what may have occurred here is that, in making the large molecular material with the theoretically adsorbed small molecule up to an equivalent total nitrogen value, the nitrogen content of the inert large molecule entered into the calculation and as a result the number of small molecules present was extremely low and gave a diminished skin reaction.

We shall probably have definite information on this subject from work now in progress in our laboratories in which we have been able to prepare pure large molecular material without contamination by adsorbed small molecules. These results will be published at a later date by another group of investigators working in our department.

SUMMARY

1. Short ragweed pollen extracts as usually prepared contain two main types of molecular aggregates roughly classified as large and small.

2. These may be separated by means of heat at the isoelectric point.

3. The small molecular aggregate has a capacity to elicit skin reactions in patients sensitive to ragweed pollen. The large molecular aggregate as prepared in the present study has this capacity but to a less marked degree.

4. The small molecular aggregate does not give precipitin reactions with ragweed precipitin antiserum. The large molecular aggregate gives positive precipitin tests with such antiserum.

5. The small molecular aggregate is incapable of eliciting anaphylactic shock in guinea pigs sensitized to whole ragweed pollen. The large molecular aggregate is capable of eliciting anaphylactic shock in guinea pigs so sensitized.

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THE RELATIONSHIP OF MATERNAL DIET TO INTRAUTERINE SENSITIZATION

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THE concept of intrauterine sensitization has led many obstetricians to practice rigid control of the maternal diet during pregnancy on the supposition that they would, in this manner, prevent hypersensitiveness in the offspring. The investigations of Ratner[1-4] in this field have received considerable attention. His concept of active or passive intrauterine sensitization is based on experimental work with guinea pigs and on a clinical study of seventeen cases of hypersensitiveness in infants that have come under his observations. Fifteen of these infants were found to be sensitive to the same foods which the mothers were said to have taken excessively during their pregnancies. Ratner[3] therefore concluded that active sensitization had taken place in utero. In another publication two cases were presented by Ratner[4] where the mothers and their offspring had reagins to the same food in their bloods. These cases were cited as evidence of passive intrauterine sensitization. Restricted diets and denatured foods were therefore recommended for the pregnant mother.

Walzer[5] in 1937 reviewed at length the question of intrauterine sensitization. He pointed out that Ratner's cases had first came under observation from six months to seven years after birth and their sensitization at birth was therefore, subject to question. Moreover all these children were breast fed for several months. As it has been demonstrated by O'Keefe,[6] Lyon,[7] Shannon,[8] Donnally,[9] and Brunner[10] that food antigens may appear in the breast milk, the possibility cannot be ruled out that these children were sensitized after birth by antigens in the mothers' milk.

The present study was undertaken to determine whether the excessive ingestion of a particular food during pregnancy would have any demonstrable effect upon the offspring from the point of view of skin reaction or sensitivity to that food.

Several hundred women attending the ante-partum clinic at Beth Moses Hospital were questioned as to their daily diet. Mothers who gave a history of excessive consumption of a particular food over a period of at least three or four months were selected as subjects for this study. The presence of a personal or family history of allergy

was noted in each case. Intracutaneous tests were performed on the newborn infant on the second and again on the eighth day with extracts of the particular food which the mother had consumed in large amounts during pregnancy. Passive transfer studies were made with the cord bloods in the majority of these cases.

TABLE I

PATIENT	ALLERGIC HISTORY		FOOD CONSUMED IN EXCESS DURING PREGNANCY	DAILY INTAKE	ALLERGIC TEST ON NEWBORN INFANT	RESULTS OF PASSIVE TRANSFER TEST
	MATERNAL	FAMILY				
B. S.	Asthma	Grandfather and aunt—hay fever	Chocolate	½-1 pt.	Chocolate	No blood obtained
M. C.	Negative	Grandfather—asthma	Milk	2-3 qt.	Milk	Negative
M. K.	Negative	Uncle—urticaria and eczema	Milk	3 qt.	Milk	Negative
K. B.	Negative	Grandfather—asthma	Chocolate	2-3 bars	Chocolate	Negative
G. K.	Negative	Negative	Chocolate	0.5 lb.	Chocolate	Negative
N. S.	Negative	Grandfather—asthma	Chocolate	2-3 bars	Chocolate	No blood obtained
L. F.	Negative	Father—asthma	Apple	4-5	Apple	Negative
J. B.	Negative	Negative	Chocolate Apple	2 bars 3-4	Chocolate Apple	Negative Negative
R. J.	Negative	Negative	Orange Chocolate	4-5 2 bars	Orange Chocolate	Negative Negative
F. M.	Negative	Negative	Chocolate	0.25-0.5 lb.	Chocolate	Negative
A. P.	Negative	Negative	Egg	3-5	Egg	Negative
R. G.	Negative	Negative	Peach Plum	3-5 4-5	Peach Plum	Negative Negative
V. M.	Urticaria	Negative	Banana Strawberry	2-4 1 cup	Banana Strawberry	Negative Negative
S. S.	Negative	Negative	Chocolate	2 bars 1 soda	Chocolate Chocolate	Negative Negative
H. M.	Negative	Negative	Apple	3 lb.	Apple	Negative
F. C.	Urticaria and hay fever	Negative	Peach Milk	4-5 2 qt.	Peach Milk	Negative Negative
P. A.	Negative	Negative	Chocolate	0.5 lb. 1 malted milk	Chocolate Chocolate	No blood obtained
B. K.	Negative	Negative	Potato	6-8	Potato	No blood obtained
A. K.	Negative	Uncle—hay fever	Buckwheat Chocolate	4-5 cakes 1 bar	Buckwheat Chocolate	No blood obtained
P. S.	Migraine	Grandmother—migraine	Chocolate	2-4 bars	Chocolate	No blood obtained
B. V.	Negative	Negative	Orange	5	Orange	Negative

Results of all direct tests were negative.

Twenty-one patients were selected and studied (Table I). Of this number, eleven gave negative personal and family histories of allergy and ten gave positive histories. The foods taken excessively by the mothers included chocolate, milk, egg, strawberry, potato, buckwheat, banana, plum, and peach. The amounts taken of each of these foods were well above the average consumption. As much as two or three quarts of milk were taken by some patients daily. Others took four to five oranges, peaches, or bananas every day. One patient ate 3 pounds of apples daily. Chocolate appeared to be a common favorite, some taking as much as 0.5 pound each day. These foods were taken excessively for three to four months because the mothers had unusual cravings for them.

Intracutaneous tests with these food extracts in routine testing strength were negative in all infants on the second and eighth days. Passive transfer studies on the cord blood obtained in fourteen of the twenty-one cases failed to show the presence of atopic reagins in the blood for any of the foods in question. The results of these studies are tabulated in Table I.

SUMMARY

1. In twenty-one cases, excessive consumption of foods, such as chocolate, milk, egg, strawberry, potato, buckwheat, banana, plum, and peach, by mothers during their pregnancies had no demonstrable effect on the offspring from a viewpoint of sensitization.

2. In the newborn infant no positive reactions to the foods consumed in excess by the mother during pregnancy were obtained by direct or indirect methods of testing.

3. From these studies as well as those of previous investigators, there does not appear to be sufficient evidence to warrant the conclusion that excessive ingestion of any food can induce sensitization in utero.

4. Hence any control of the maternal diet during pregnancy aimed at the prevention of intrauterine sensitization does not seem justified.

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PROPHYLACTIC ORAL THERAPY AGAINST POISON IVY

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IN THE spring of 1941 following publication of Shelmire's work on oral poison ivy therapy[1] we attempted to study this problem in a group of twenty subjects known to be susceptible to poison ivy. They were inmates of the Elwyn Training School and included seventeen males and three females. Their ages ranged from 10 to 34 years. Seventeen of the patients were under 18 years of age.

The procedure followed in our study consisted of (1) initial patch test, (2) oral therapy, (3) repatch test seven to fourteen days after the last dose, (4) additional oral therapy if indicated, (5) final patch test, and (6) field test (exposure to poison ivy plants).

The patch test was performed as follows. A drop of poison ivy extract of varying strength* was applied on the skin of the arm, forearm, or back with the end of a wooden applicator. The drop was then covered with a small piece of absorbent paper and overlaid with a larger square of cellophane that was held in place for twenty-four hours by means of adhesive plaster. Thus we were able to secure a distinct area of dermatitis easily differentiated from the irritative changes caused by the adhesive. The patch test reactions were observed for seven days. They were artificially graded as + when they consisted of mottled erythema, ++ when they appeared as solid erythema with swelling of the skin, and +++ when there was added vesiculation. It was noted that the length of time required for the appearance of the patch reaction and its flowering was directly proportional to the degree of skin sensitivity and concentration of the extract.

The same extracts were used in subsequent patch tests, but different sites were selected to avoid either refractoriness or hypersensitivity of the skin due to previous reactions. In each instance, the potency of the extracts was controlled by simultaneous patch testing of untreated sensitive patients.

Treatment consisted in swallowing poison ivy tablets prepared for us by the Mulford Biological Laboratories, Sharp and Dohme.† The tablets contained 0.5, 2.0, 5.0, 10.0 mg. of poison ivy oleoresin each. The

*The extract was prepared as follows. Fresh leaves were gathered, cut into small pieces, and packed in a percolator. The leaves were covered with ethyl ether and were allowed to remain in contact with this solvent for about one week. The extract was collected and the solvent evaporated off at room temperature. A thick semisolid, black, tarlike material was recovered.

For patch test purposes, dilutions of this stock preparation were made with corn oil.

†"Ivagen" poison ivy tablets, Sharp and Dohme.

required number were swallowed right after the noon meal under our direct supervision. The initial dose was one-half to one 0.5 mg. tablet. Daily treatment was given unless severe reactions occurred, when it

TABLE I

PROPHYLACTIC ORAL THERAPY AGAINST POISON IVY

	DAILY INTAKE	
	NO. OF TABLETS	MG.
1	1	0.5
2	2	0.5
3	4	0.5
4	6	0.5
5	2	2.0
6	3	2.0
7	4	2.0
8	2	5.0
9	2	5.0
10	2	5.0
11	2	5.0
12	2	5.0
13	2	5.0
14	2	5.0
15	2	5.0
	1	2.0
16	3	5.0
17	4	5.0
18	5	5.0
19	3	10.0
20	4	10.0
21	5	10.0
22	6	10.0
23	7	10.0
24	8	10.0
25	10	10.0
26	10	10.0
27	10	10.0
28	10	10.0
29	10	10.0
30	10	10.0
31	10	10.0
32	10	10.0
33	10	10.0
34	10	10.0
35	12	10.0
36	15	10.0
37	15	10.0
38	15	10.0
39	15	10.0
40	15	10.0
41	15	10.0
42	15	10.0
43	15	10.0
44	15	10.0
45	15	10.0

was given every second day or at longer intervals as indicated. The dosage schedule used in this experiment is given in Table I. The maximum dose reached in all patients but one was fifteen tablets, 10.0 mg. each, or a daily total of 150 mg. of oleoresin. The exception received the enormous dose of 500 mg. per day without untoward effects.

REACTIONS FROM ORAL THERAPY

A variety of skin eruptions was encountered in all but four of our patients. They consisted of punctate or solid erythema, patchy or diffuse in distribution, that occurred most often on the arms and chest and was accompanied by pruritus. In some a fine papular rash developed. In two subjects there occurred a vesicular dermatitis of the hands with oozing. In only one subject did a localized dermatitis above the wrist develop the characteristics of a fixed rash that did not disappear until the end of oral therapy. When the dermatitis was severe, the patient was given the oleoresin tablets every second or third day, or the treatment was stopped for a few days and then restarted at the last dose level without encountering further difficulty. Actually upon continued and increasing oral therapy, most of the skin eruptions subsided within a few days. In many, recovery was associated with local desquamation. As Shelmire has pointed out,[1b, c] these skin reactions are of the toxic medicamentosa type and are probably due to overburdened tolerance. An analysis of their time occurrence discloses the fact that practically all of them developed between the second or third and the twelfth to fourteenth days of treatment. Undoubtedly they were due to too rapid an increase in dosage that led to too great a concentration of ivy oleoresin in the blood before a suitable tolerance had developed. Their incidence can be greatly decreased by a more gradual increase in dosage spread out over a longer period of time. While suffering from toxic dermatitis, two of our subjects contracted measles and were very sick. This may have been coincidental, although the rest of the cases of measles that occurred in the same pavilion were of a mild type. Complete recovery ensued.

Pruritus ani occurred in five subjects. In three of them it was very annoying and persisted throughout the course of treatment (these patients failed to develop protection against poison ivy). In the other two it reappeared occasionally whenever their tolerance was overburdened by too rapid an increase in dosage. There was no perianal dermatitis, but only traumatic evidences of scratching.

Notwithstanding the large doses of poison ivy oleoresin administered, there were no gastrointestinal upsets and no urinary complaints. Repeated blood counts and urine studies failed to show any abnormalities.

DOSAGE AND RESULTS OF TREATMENT

In order to determine the minimum total amount of oleoresin that must be ingested to alter the skin reaction to poison ivy, it was decided to administer varying doses (Table II). Group A consisted of two subjects (Patients 1 and 2) who received 1,496.75 mg. each; Group B, of five subjects (Patients 3 to 7 inclusive) who received 2,134.75 to 2,239.75 mg. each; Group C, of two subjects (Patients 8 and 9) who received 2,904.5 and 2,924.5 mg. respectively; Group D, of seven sub-

jects (Patients 10 to 16 inclusive) who received from 3,064.25 to 3,144.25 mg. each; Group E, of four subjects (Patients 17 to 20 inclusive) who received from 3,224.25 to 3,357.25 mg. each.

Patch tests with 1:100 dilution of the oleoresin were done seven to fourteen days after the last dose (Table II).

The two members of Group A and three members of Group B showed a decrease in skin sensitivity. The other two patients (Group B) showed little if any change. The seven patients were then given an additional course of therapy. Group A received 1,620 mg. in eleven days while Group B was given 500 mg. in ten days. Recheck patch tests showed a further decrease in skin sensitivity in Group A and in two members of Group B who had previously shown no change. In the other three subjects there was no change in the skin reaction. The results obtained after the two courses of oral therapy would indicate that a decrease of the skin sensitivity to poison ivy occurred in these seven subjects.

Of the nine members of Groups C and D, three showed negative reactions on recheck patch test, three gave questionable reactions, two gave one-plus reactions, and one subject showed a two-plus reaction (test performed on the third day after onset of clinical poison ivy). This last patient (Subject 16) was interesting because he developed poison ivy dermatitis nine days after the last dose before a recheck patch test was done. This attack of poison ivy was only moderate in severity and lasted about a week without the use of any local medication. This was in marked contrast to the experience of the preceding two years when the patient had to be admitted to the hospital for ten days, twice each season because of severe poison ivy dermatitis with vesiculation and marked edema of the face and extremities. Although the degree of exposure that precipitated the present dermatitis was not known, it was apparent to all the medical attendants acquainted with this boy's past history of marked sensitivity to poison ivy that he was now enjoying a partial immunity against the disease. After recovery, he was given an additional course of treatment; 2,880 mg. of oleoresin were ingested by him in a period of ten days. The first dose of 150 mg. was similar to that received at the end of the first course (nineteen days elapsed between the two) while the final dose consisted of 500 mg. of the oleoresin. A recheck patch test done thirteen days later evoked a one-plus reaction, denoting a further decrease in skin sensitivity.

It would appear that following oral treatment (Table II) the nine subjects of Groups C and D showed a decrease in skin sensitivity, with complete obliteration in three and almost complete disappearance of skin reaction in three additional patients. The difference between these results and those obtained in Groups A and B can be accounted for

TABLE II

PROPHYLACTIC ORAL THERAPY AGAINST POISON IVY

SUBJECT	GROUP	CONTROL PATCH TEST		FIRST COURSE DOSE (MG.)	RECHECK PATCH TEST		FINAL TOTAL DOSE (MG.)	FINAL PATCH TEST		FIELD TEST
		DILUTION	RESULT		DILUTION	RESULT		DILUTION	RESULT	
1	A	1:1,000	++	1,496.75	1:100	++	3,116.75	1:100	+	N.P.[2]
										P.P.[3]
2	A	1:1,000	+	1,496.75	1:100	+	3,116.75	1:100	?	P.P.[2]
										G.P.[3]
3	B	1:1,000	++	2,134.75	1:100	+	3,634.75	1:100	+	C.P.[3]
4	B	1:1,000	+	2,239.75	1:100	++	3,739.75	1:100	+	G.P.[3]
5	B	1:1,000	+	2,239.75	1:100	+	3,739.75	1:100	+	C.P.[3]
6	B	1:1,000	++	2,229.75	1:100	+++	3,729.75	1:100	++	P.P.[3]
7	B	1:1,000	+	2,239.75	1:100	+	3,739.75	1:100	+	G.P.[3]
8	C	1:100	+	2,904.5	1:100	0				G.P.[2]
										C.P.[3]
9	C	1:100	+	2,924.5	1:100	?				C.P.[1-3]
10	D	1:100	++	3,144.25	1:100	+				C.P.[1-3]
11	D	1:100	++	3,094.25	1:100	0				C.P.[1-3]
12	D	1:100	+	3,104.25	1:100	0				C.P.[1-3]
13	D	1:100	++	3,024.5	1:100	+				C.P.[1]
14	D	1:100	++	3,094.5	1:100	?				C.P.[1-3]
15	D	1:100	+	3,064.25	1:100	?				C.P.[2]
16	D	1:100	+++	3,104.25	1:100	++	5,984.25	1:100	+	G.P.[3]
17	E	1:100	++	3,224.25	1:100	+				G.P.[3]
18	E	1:1,000	++	3,312.25	1:100	+++				N.P.[3]
19	E	1:1,000	+++	3,357.25	1:100	+++				N.P.[3]
20	E	1:1,000	+	3,328.75	1:100	+++				N.P.[3]
				Controls						
21		1:100	+++							N.P.[1-2]
22		1:100	++							N.P.[1]
23		1:100	++							N.P.[1]
24		1:100	++							N.P.[2]
25		1:1,000	++							N.P.[3]
26		1:1,000	+							N.P.[3]
27		1:1,000	++							N.P.[3]
28		1:100	+++							N.P.[3]

+, Mottled erythema; ++, solid erythema with swelling; +++, solid erythema with swelling and vesiculation.
C.P., Complete protection; G.P., good protection; P.P., partial protection; N.P., no protection.
[1]Field exposure No. 1; [2]field exposure No. 2; [3]field exposure No. 3 (see text for description).

on the basis that the members of the latter two groups were more sensitive to poison ivy before treatment was given.

Of the four members of Group E, one showed a definite decrease in skin sensitivity, while the other three showed no change. Actually these three subjects (Subjects 18 to 20 inclusive) were the only ones in the entire series who showed a three-plus reaction (vesiculation) following treatment.

According to the results of the patch test, it would appear that, of twenty subjects who underwent prophylactic oral therapy, seventeen showed some decrease in the skin sensitivity. This decrease was expressed not only in terms of lessened intensity of reaction (lack of vesiculation and edema, mottled erythema, etc.), but also in a lengthened interval of time that elapsed between the removal of the patch test and the onset of the allergic reaction, and the shorter duration (early involution) of the latter.

Although individual variation is marked, it appears that a total dosage of about 3,000 mg. of poison ivy oleoresin must be ingested to obtain a significant decrease in skin sensitivity. In the majority of our cases the decrease in skin reactivity seems to bear a direct relationship to the total amount of oleoresin ingested. However, this does not rule out the possibility that the size of the final individual dose rather than the size of total dosage ingested determines the degree of desensitization that occurs. In this regard we must also consider the possible influence exerted by the higher oleoresin concentration in the blood that results from the cumulative effect produced by the repeated ingestion of poison ivy material.

FIELD TESTS AND RESULTS

Since actual controlled exposure to poison ivy is the only effective way of determining whether immunity exists, we attempted to check on the value of prophylactic oral therapy by subjecting our patients to a field test. In devising a field test one must remember that the degree of exposure or intimacy of contact is an important factor in determining the severity of the specific dermatitis that may ensue. It is quite possible that no amount of oral therapy will prevent the occurrence of dermatitis if the field test is too severe. We, therefore, decided on three types of field tests which were carried out under our direct supervision, with proper identification of the poison ivy plants, suitable controls (exposure of untreated susceptible persons), and adequate follow-up for seven to ten days.

Field Test No. 1—The patient walked a distance of about 15 feet in a semicrouched position in a field of poison ivy in search of a rubber ball. The legs and forearms were uncovered. Close contact with poison ivy leaves was observed. As the patient emerged from the field, he was

asked to pull three or four ivy plants after which he returned to his regular routine without washing the hands or changing clothes and shoes.

Field Test No. 2.—The patient walked into a honeysuckle field overgrown with poison ivy. The legs and arms were uncovered. Under direct supervision the patient pulled up the poison ivy plants until a handful was collected (roots included). He was then told to rub it firmly against the opposite forearm (flexor surface). Finally, both forearms were rubbed against each other.

Field Test No. 3.—This test was similar to No. 1 but at the end, instead of pulling up the plants, the patient was asked to lie down in the ivy field for a brief moment.

Some of the patients were subjected to more than one field test. The results were tabulated as no protection (N.P.) when the treated subjects had diffuse dermatitis as intense as that of the controls; partial protection (P.P.) when localized nonvesicular dermatitis occurred, which healed in a few days; good protection (G.P.) when an occasional discrete papule or small patch of erythema occurred here and there; and complete protection (C.P.) when no dermatitis developed.

Six subjects (Subjects 9 to 14 inclusive) underwent field test No. 1 (Table II). All of them had complete protection. Patch tests (1:100) performed before the field test showed that two of the patients had a one-plus reaction, while the rest were negative. The three controls exposed to field test No. 1 developed poison ivy within twenty-four hours. In one the dermatitis was severe; in one it was moderate; and in the other it was slight. The slight dermatitis actually occurred in the most sensitive subject (three-plus patch test, 1:100). However, this patient was very uncooperative and had the least exposure to the plants. Six weeks later, five of the treated subjects resubmitted to field test No. 3 with identical results.

Four patients (1, 2, 8, 15) were subjected to field test No. 2 (Table II). Subject 1 had a one-plus patch test (1:100) before exposure, but he showed no protection. The other three, who had negative skin patch tests, showed partial, good, and complete protection, respectively. That field test No. 2 was the most severe of all is proved by the fact that, when three of the treated subjects were induced to undergo field test No. 3 five weeks after the performance of field test No. 2, they all showed a greater degree of protection (No. 1 from N.P. to P.P., No. 2 from P.P. to G.P. and No. 8 from G.P. to C.P.). Similarly, the two controls for field test No. 2 developed severe dermatitis, and one of them was the patient who, on field test No. 1, had contracted only a slight dermatitis. That the better results obtained under the conditions of field test No. 3 were due to lessened exposure was further proved by the results of patch tests performed on two of the treated subjects

(Subjects 1 and 2) three weeks before the second field test No. 3 was carried out (two weeks after the dermatitis occurred as a result of the first field test No. 2). At this time the patch tests indicated an increased reactivity of the skin, from + to ++ and from questionable (?) to +, respectively, yet the patients had shown greater protection against poison ivy during field test No. 3.

In addition to the eight patients already mentioned, ten members of our series were subjected to field test No. 3 (Table II). Two showed complete protection; four, good protection; one partial protection; and three, no protection. There was good correlation between the results of the patch tests and the field tests in all the patients. The four controls developed poison ivy. The three subjects who developed contact dermatitis as severe as those of the controls (N.P.) were three girls who had toxic dermatitis and persistent pruritus ani throughout the oral therapy. This confirms Shelmire's observation of the value of pruritus ani and its disappearance as an indicator of developing tolerance to poison ivy.

This incidence of failure, three cases of twenty, is also in agreement with the experience of Shelmire,[1c] who stated that the "results of oral therapy paralleled those usually attained in the percutaneous treatment of the asthma and hay fever patient." Our failures were certainly not due to low total dosage since they were members of Group E who had received the largest amount of oleoresin.

The conditions governing our field tests were definitely more severe than those found under the ordinary everyday exposure responsible for poison ivy dermatitis. Hence we feel that seventeen of our subjects developed adequate protection as a result of the oral therapy they received.

COMMENT

Shelmire has pointed out that past attempts at immunization against poison ivy have failed because of the use of (1) extracts that were not potent and (2) inadequate dosage. Unfortunately it is impossible to administer parenterally an adequate dose of a potent extract. The largest amount that we have been able to inject subcutaneously has been 0.5 c.c. of a 1:50 solution, the equivalent of 10 mg. of oleoresin, and this invariably produced severe local reactions. Hence the value of oral desensitization.

Our findings serve to confirm those of Shelmire. In addition, we feel that, by administering the oleoresin in tablet form, we removed the danger of contact dermatitis of the hands and mouth which may result from the patient handling and swallowing the liquid preparation as advised by Shelmire.

Moreover, our tablets have thus far retained their potency over a period of 2.5 years. It is a well-known fact that the percentage of active principle will vary from plant to plant and from season to season. In the absence of a chemical test to determine the activity of various extracts, extensive patch testing will be required to evaluate potency. Hence the schedule of treatment and observations made by us apply only to the tablets used in this experiment.

This is a preliminary study. Large, well-controlled clinical trials of this prophylactic measure should be done to determine its usefulness on an accurate statistical basis.

SUMMARY

1. Prophylactic oral therapy against poison ivy was administered to twenty susceptible persons.

2. The dose, reactions, and results of patch tests and field exposures are described.

3. Seventeen subjects responded to the oral treatment. Three were considered failures.

We wish to thank Dr. E. A. Whitney, Medical Director of the Elwyn Training School, Elwyn, Pa., for permission to carry out this study. Mrs. Sara Michael of the Elwyn School staff gave us valuable assistance.

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314 E. 9TH ST.

THE CEREAL-FREE ELIMINATION DIETS AND THE SOYBEAN EMULSION FOR THE STUDY AND CONTROL OF INFANTILE ECZEMA

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THE use of a cereal-free elimination diet modified for infants has indicated for several years the frequency of food allergy as a cause of infantile eczema. Foods in the diet to which definite scratch reactions occur should be replaced by negatively reacting foods. It must be remembered, however, that positive reactions are not necessarily associated with clinical allergy.

CEREAL-FREE ELIMINATION DIET FOR INFANTS (ROWE)[1]

Soybean cereal-free formula (see directions below).

Mull-Soy (Muller Laboratories, Baltimore, Md.). (Dilute with equal amount of water.)

Kreme O'Soy (Madison Foods, Madison College, Tenn.).

Sobee (Mead Johnson & Co.); 26 to 27 tablespoonfuls in 7 ounces of water boiled for three minutes.

Feedings of 5 to 8 ounces every four hours according to the infant's caloric requirements. Two to four tablespoonfuls of cane sugar as tolerated may be added to the formula for the day. Similar amounts of dextrimaltose may also be used.

Sobee cooked with water for one-half hour may be used as a cereal, but the presence of arrowroot flour and olive oil in Sobee must be remembered.

If soybean allergy or intolerance exists, Cemac (Mead Johnson & Co.) containing homogenized beef, vegetables, calcium, phosphorus, and iron may be tried.

For vitamin A, give Caritol in sesame oil, 8 to 10 drops daily.

For vitamin B_1 and B_2 give, in feedings, the contents of 2 B complex capsules each day.

For vitamin C, give cevitamic acid, 50 mg. daily.

For vitamin D, give viosterol in sesame oil or Drisdol, 8 to 10 drops daily.

Adequate calcium and phosphorus are contained in the above soybean preparations.

To the initial elimination diet, the following foods should also be added according to the infant's age, desire, and digestive and allergic tolerance:

Grapefruit juice, sweetened, from the first to the third month. Tomato juice may also be given if tolerated.

Puréed spinach, carrot, asparagus, beet, peas, string beans, Lima beans, artichoke, or tomato from the third to fourth month. (Trial of one or two at a time will evidence any allergic reactivity.)

Soft cooked white or sweet potato from the third to the fifth month.

Puréed pear, peach, apricot, or prune from the third to the sixth month.

Tapioca cooked with a little puréed fruit and sugar from the fourth to the sixth month.

Scraped or finely minced lamb or beef and also bacon from the eighth to the tenth month.

Butter substitutes such as Sesame Spread containing hydrogenated sesame oil with artificial color and salt or hydrogenated sesame oil may be used. (Nu-Spread Co., Portland, Oregon.)

Salt, cane or beet sugar, and sesame oil may also be used.

After relief is definite, the following foods may be added, one every five to ten days:

Orange, lemon, pineapple, apple, banana, cow's milk (evaporated or dried and later pasteurized), cream, butter, rice, corn, rye, wheat, chicken (no hens).

Eggs, chocolate, fish, and nuts should be given to the child with atopic dermatitis only during the second year.

THE SOYBEAN EMULSION AS A SUBSTITUTE FOR COW'S MILK

A soybean emulsion is a valuable substitute for cow's or goat's milk since this bean is the only vegetable source of all amino acids required by human beings. The commercial canned products suggested in the above diet are acceptable to most infants and maintain nutrition. The following formula can be prepared at home inexpensively. It has been developed from one originally suggested by Dr. Katherine Bain.* It contains foods allowed in the writer's cereal-free elimination diet, and the carbohydrate, protein, fat, calcium, or phosphorous content is comparable to that of cow's milk (Table I).

Directions for Mixing

All measurements are level and packed. Use standard tablespoon (Tbs.) and teaspoon (tsp.) for measuring.

1. Put the soybean oil in the top of a double boiler. Heat and add arrowroot or tapioca flour to make a smooth paste.

*Assistance by Eva Court, dietitian, was given in the preparation of the formula herein described.

TABLE I

SOYBEAN CEREAL-FREE FORMULA (ROWE)*

	MEASURE	GRAMS	CARBOHYDRATE (GM.)	PROTEIN (GM.)	FAT (GM.)	CALORIES	CA	P	FE	NaCl
Soybean flour†	11 Tbs.	72.00	5.8	32.4	7.9	223	0.155	0.419	1.94	
Soybean or sesame oil	3 Tbs.	30.00			30.0	270				
Tapioca or arrowroot flour‡	3 tsp.	10.00	10.0			40				
Sugar	3 Tbs.	32.00	32.0			128				
Dicalcium phosphate	1¼ tsp.	4.30					1.000	0.774		
NaCl	¼ tsp.	1.68								1.68
Water to make	32 oz.		47.8	32.4	37.9	661	1.155	1.193	1.94	1.68
The equivalent amount of whole milk contains:										
Whole milk	32 oz.	960	48.0	31.6	38.0	664	1.52	0.893	1.92	1.68

*Revised from a soybean flour formula suggested by Dr. Katherine Bain and Miriam Lowenberg.

†Staley's Soy Flour No. 1 (A. E. Staley Manufacturing Co., Decatur, Ill.) or Nutri Soy Flour No. 3 or 5 (Archer Daniels Midland Co., Minneapolis, Minn.) or similar processed soybean flours make a smooth, nonprecipitating emulsion. Raw soybean flour produces an unsatisfactory product, and its protein is precipitated as a "curd" when the calcium salts are added, especially by the more soluble calcium gluconate.

‡Tapioca flour is less expensive than arrowroot flour and is obtainable at baker's supply companies. If 4 teaspoonfuls of tapioca or arrowroot flour and 2⅔ tablespoonfuls of sugar are used, a thicker emulsion results with the same carbohydrate content.

Carbohydrate, protein, and fat values were obtained from Bridges, M. A.: Food and Beverage Analyses, Philadelphia, 1935, Lea & Febiger. Calcium, phosphorus, and iron values were obtained from Bowes and Church: Food Values of Portions Commonly Used, Philadelphia, 1940, Child Health Society.

2. Add sugar, salt, dicalcium phosphate to 1 cup of hot water. Mix this with the soybean flour to make a smooth paste, free from lumps. Add the extra water, to make 32 oz.

3. Add this mixture to No. 1 over the flame, stirring constantly. After the boiling point has been reached, cook for a few minutes. Replace kettle over the lower half of the double boiler. Cook for twenty minutes.

COMMENT

We agree with Levin[2] that soybean-milk emulsions as recommended in this article are better tolerated and are more palatable than is Sobee. Cane sugar has also been used in our cereal-free diet. Occasional intolerance or possible allergy to sugar has seemed likely in an occasional case. Allergy to soybean as reported by Levin and others has occurred in a few of our patients. Modified animal milks may be tried in such instances. Recently homogenized, liquefied lamb, beef, and liver have been prepared and may be of value when soybean allergy exists.

In addition to diet trial, the value of the other recommendations of Levin has been confirmed in our practice. The proper environmental control, the adequate treatment of the varying degree of seborrhea

which is present in most patients, the elimination of woolen blankets which mechanically irritate the skin, bathing the infant three or four times a week with soap and water, and the use of crude coal tar ointment when a dry scaly eczema is present and a calamine lotion to which phenol may be added when a moist eruption is present are of definite importance. Proper vitamin therapy as suggested above in the dietary recommendations is necessary.

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2940 Summit St.

Special Reports

UNUSUAL HYPERSENSITIVITY TO HOUSE DUST IN A CASE OF ALLERGIC CONJUNCTIVITIS

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HOUSE dust is one of the most usual allergens responsible for allergic conjunctivitis. Vaughan[1] found such patients more often sensitized to house dust and foods than to pollen. Unger[2] noted the lack of seasonal relation between hay fever and vernal conjunctivitis and stated that many cases of vernal catarrh give negative skin tests and yet their symptoms come on year after year with the hot weather. Bowen[3] classified allergic conjunctivitis into two types, the vernal and the nonvernal. The vernal type, he considered a form of contact dermatitis. Lehrfeld,[4] working originally with scratch tests and later by intradermal technique, found that the majority of his patients were sensitive to house dust and that no incidence of the limbic type and the lid type occurred coincidentally in the same patient.

The following case report seems of interest in that a proved house dust sensitivity was found in a presumably nonatopic person who gave negative scratch and intradermal skin reactions to all of the usual allergens. It is of further interest that the condition occurred during the winter, that both the lids and ocular conjunctivae were affected, and that a negative intradermal skin test with 0.02 c.c. of a 1:50 dialyzed aqueous dust extract was followed by a general anaphylactic reaction.

CASE REPORT

S. W., a student nurse, aged 21 years, was admitted to the Louisville City Hospital, Jan. 12, 1941, complaining of acute conjunctivitis. The eyelids and bulbar conjunctiva were infected, but there was no evidence of purulent discharge. There was no previous history of eye disease, refractive error. neurasthenia, or allergic conditions.

The physical examination, except for the eyes, was essentially normal. The patient's mother had had asthma. Otherwise, there was no allergic family history.

The patient's eyes were treated with boric acid irrigations and instillations of argyrol solution. Her discomfort was partially relieved by instillations of 4 per cent cocaine solution and the ingestion of codeine, and aspirin. The blood count showed 12,500 white cells, 5 per cent of which were monocytes, 2 per cent eosinophiles, 17 per cent lymphocytes, 76 per cent neutrophiles. On January 16, the temperature rose to 100° F., and evidence of acute pharyngitis was noted. Her condition improved rapidly, and she was discharged Jan. 19, 1941.

Three days later, the patient was readmitted to the hospital complaining of headache and conjunctivitis. The temperature was normal. Smears taken from the conjunctiva showed no organisms. A differential count of a Wright stained conjunctival smear showed 85 per cent eosinophiles. The eyelids were painted with a 2 per cent solution of silver nitrate; eye irrigations were resumed; and instillations of aptochin base were prescribed. The patient was discharged to the nurses' home after two days of hospitalization and was told to report to the Allergy Clinic and to the Outpatient's Eye Dispensary. The patient was readmitted to the hospital Jan. 31, 1941, with a recurrent acute catarrhal bilateral conjunctivitis. *Streptococcus viridans* was isolated in culture from the conjunctivae. On February 1, the patient was discharged with the notation on progress stating that the conjunctivitis had cleared up entirely. Skin tests made in the Allergy Clinic on January 23, to foods, fungi, and the usual inhalants were negative. Patch tests to cosmetics and drugs to which the patient was exposed were likewise negative.

On Feb. 4, 1941, at 11.30 A.M. an intradermal skin test of 0.02 c.c. of a 1:50 dialyzed aqueous extract of house dust was done and reported as negative. The patient stated that she became short of breath about one hour after leaving the clinic. She was readmitted to the hospital at 4:00 P.M. suffering a severe type of hyperpnea. Her respirations were described as extremely shallow. A latter progress note stated that the patient was having a typical tetany brought on by carbon dioxide loss. An oxygen tent and calcium and viosterol therapy were prescribed. The right eye showed slight infection, cultures of which showed *Staphylococcus aureus*. A roentgenogram of the chest was negative. The patient was discharged February 13.

On February 18, the patient appeared in the Allergy Clinic and was wearing protective glasses. The conjunctivae were markedly improved. A subcutaneous injection of 0.05 c.c. of undialyzed aqueous house dust extract was given. No local or general reaction was noted after thirty minutes. The patient stated that about one hour after the injection, she experienced some asthma which subsided without medication. On February 25, she received a subcutaneous injection of 0.05 c.c. of a 1:100 dilution of undialyzed house dust. She experienced no local or general reaction.

On March 4, undialyzed stock dust, diluted 1:1,000 with saline was instilled in the patient's right eye, and physiologic saline solution in the left. In seven minutes, the right ocular conjunctiva became reddened, and the patient complained of itching in this eye. The same 1:1,000 dust extract gave no reaction in control eyes. Scratch skin tests made with extracts from the patient's mattress and room dust were negative. Two positive passive transfer tests were recorded from the patient's serum on two normal controls. The patient continued to receive increasing amounts of dust extract at weekly intervals, without untoward symptoms.

On May 2, the patient received 0.1 c.c. of a 1:10 extract of undialyzed dust and the notation made that she was entirely relieved of conjunctivitis.

COMMENT

This patient presented an unusually severe type of conjunctivitis, which probably was due to both allergy and infection. The positive intraocular test and positive passive transfer tests in the absence of positive skin tests are of interest.

The tetany suffered by the patient is difficult to explain on the basis of anaphylaxis since an asthmatic state would probably decrease the

blood oxygen. The patient, however, was a most stable type and in her three years as a student nurse had shown no signs of neurosis or hysteria.

The improvement in the eye condition which followed the wearing of occlusive glasses is suggestive of contact dermatitis, but, after receiving dust injections, the patient remained free of symptoms without the use of other supportive therapy.

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CEREBRAL ALLERGIC EDEMA

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VARIED symptom complexes due to cerebral edema caused by allergic phenomena have been recorded. In 1927 Vaughan[1] definitely connected food allergy with Galen's hemicrania. That an allergic edema of the internal ear can produce vertigo has been proved by Criep[2] and Clark.[3] Certain types of epilepsy have been given an allergic basis.[4] Further comprehensive work of headaches of allergic origin has been furnished by Thomas and Johnston.[5] Choreiform movements, marked personality changes, and mental backwardness in the allergic person with improvement following proper desensitization have been seen.[6]

This case of probable cerebral edema is of interest because it reveals the importance of considering cerebral allergy in differential diagnosis of diseases of the central nervous system and at the same time offers an explanation of the unusual behavior and personality found in the patient.

CASE REPORT

W. R. S., Jr., a white male, aged 16 years, was first seen at the Good Samaritan Clinic in 1933, when he was 9 years old. He was undersized and had not grown during the four preceding years. Dull in school, he was then making the fourth annual attempt to pass the first grade. This mental inaptitude was explained by an almost diurnal sleepiness, accompanied by an occasional dull, boring pain between the eyes, since its onset in 1930. He was very nervous, was never still, had a poor appetite, and awoke every morning feeling very cold.

At birth, which had been normal in every way, he weighed 8 pounds. His earliest development was uneventful until the beginning of the headaches in January, 1930. He had had an uncomplicated diphtheria treated with antitoxin at 2 years, measles at 7 years, and an occasional epistaxis without any rheumatic involvement. His mother and two maternal uncles had asthma.

Examination revealed normal height and weight for his chronological age and a retardation of his mental age with an I.Q. (Sanford-Binet) of 71, low normal being 90. Other findings included dry, coarse skin and hair, infected tonsils, enlarged epitrochlears, and a palpable thyroid gland. The testes, although descended, were smaller than normal. The basal metabolic rate was minus 12 per cent. The Schilling differential count disclosed an eosinophilia of 12 per cent. The blood Wassermann test was negative.

Treatment for hypothyroidism and hypogonadism was instigated. In December, 1933, a tonsillectomy was performed. For the next six years the patient received thyroid extract and orchic substance. His height increased 9.5 inches, but the intelligence improved only slightly. All medication was stopped on July 5, 1938. In

From the Good Samaritan Clinic, Atlanta.

the interim, he had passed the fourth grade requirements, although the sleepy spells had increased in frequency, with as many as three to twenty a day, lasting from five to sixty minutes. He was advised to quit school and take up a trade.

In March, 1940, I saw the patient for the first time. His father, as informant, stated that, for the past seven years, his son had complained of severe, one-sided frontal headaches, which were occasionally heralded by a swelling of the lips and beneath one eye. These headaches, without aura or other prodromata, were always associated with drowsiness and sometimes with nausea and vomiting. First, the facial swelling appeared, soon to be followed by headache; then, in about an hour came increasing drowsiness. If permitted, the patient would sleep but could be aroused by vigorous shaking. The lethargy usually cleared in ten or twelve hours, but the swelling required two or three additional hours for its disappearance. The attacks had no seasonal variation, began at any time of the day, and occurred from one to three times a month. Neither patient nor informant had discovered any precipitating factors.

Re-examination revealed a well-developed but mentally sluggish 16-year-old male with moderate edema of the lips. The pulse rate was 78 per minute; the blood pressure, 115/70 mm. in both arms. The hair and skin were dry. The thyroid was of normal size and consistency. The genitals were normally developed. A 4 per cent eosinophilia was found in the blood. The rest of the blood count, the blood chemistry, urinalysis, phenolsulphonephthalein test of kidney function, and the feces were all normal. The blood Kahn was negative. X-ray examination of the head showed no pathology. The basal metabolic rate was minus 21 per cent with a pulse rate of 68 per minute. Because of the familial history of allergy and the migrainous-like headaches with facial edema associated with drowsiness, cerebral angioneurotic edema with possible hypothalamic involvement was strongly suspected. Dr. Clarence Laws made both scratch and intradermal skin tests, which showed sensitization to the following foods: corn, rice, wheat, spinach, beans, peas, apple, pear, orange, grapefruit, lemon, and all seasonings.

Neurological examination by Dr. Richard Wilson revealed occasional spasmodic contractions of the head and limbs. At times, only one limb was involved, but occasionally all were abducted to a slight degree. No objective evidence of organic disease of the central nervous system was found. Dr. Wilson could explain his findings only on the basis of a cerebral angioneurotic edema which probably involved predominantly the hypothalamus. Mr. Robert Brush, a psychologist, using the Wechsler-Bellevue intelligence test, rated the patient's total I.Q. at 50, which suggested definite mental deficiency. His isolated vocabulary rating was 72. Since March, 1940, when the offending foods were omitted from the diet, the patient has remained free of attacks of drowsiness and facial edema. In May, 1940, the patient was given purposely a piece of wheat bread. Forty-five minutes after ingestion, periorbital edema of the right eye occurred. One hour later, the patient became very lethargic. Adrenalin chloride (1:1,000 solution), 0.2 c.c., was given every ten minutes for four doses; the edema and drowsiness almost cleared within the next hour. There have been no recurrences since this experimentally induced one. The patient has now shown greater aptitude in carrying out his present job as a mechanic, than at any previous time.

DISCUSSION

The cerebral pathology involved can best be understood by reviewing the dog experiments of Davidoff and Kopeloff.[7] They sensitized a previously injured left cerebral motor area with horse serum and two to four weeks later administered horse serum, intravenously, to these

dogs. A transient right-sided hemiparesis ensued. While injecting the brains of the animals, they accidentally sensitized the overlying scalp, which reacted along with the cerebral tissue and, in so doing, served as a visual reaction indicator. This is quite analogous to the periorbital and cerebral edema observed in the present case. The cerebral edema, with foods as the excitant allergens, is best explained by local vasodilatation and increased capillary permeability.[5] That the hypothalamus was a most vulnerable area in this case is very suggestive. Improvement in personality on subsidence of frontal lobe edema is a likely possibility. Kennedy and his associates[8] considered vasomotor changes in cerebral vessels as an important mechanism in epilepsy, arterial hypertension, carotid sinus syncope, migraine, and angioneurotic edema Migraine was associated with vasodilatation, a beginning rise in a fallen blood pressure, and an increased amplitude of intracranial pulsations when the headache was severest. Kennedy observed the exposed cortex of an epileptic person undergoing operation with local anesthesia. He described a primary cortical blanching followed by marked venous engorgement and brain protrusion, at the onset of the attack. Carotid sinus syncope was explained on the basis of a momentary constriction and then marked dilatation of the pial vessels. Sudden cerebral anoxemia produced in this way was held as a likely explanation of the vagal and depressor carotid sinus response.

Goltman's[9] observations on allergic migraine in a young woman were greatly sustained by a postoperative opening in the left frontal area of the skull. He concluded that the mechanism of migraine consisted of a primary vasomotor spasm, a secondary vascular dilatation with resulting edema of the brain. A subsequent state of hypersecretion along with a simultaneous hyperabsorption of the cerebrospinal fluid brought an eventual equalization of pressure in the subarachnoid space. This was successfully controlled by avoidance of the causative irritants, which were both foods and inhalants.

Cerebral allergy can simulate a countless number of diseases. There are, on record, cases where it has effected marked personality and mental changes.[6] With improvement in our diagnostic apparatus, along with continued detailed study of each case, we may be able to recognize the underlying altered physiology in these unfortunate people and thereby establish them as useful citizens in their community.

SUMMARY

1. A case of food allergy causing cerebral edema in a patient with familial allergy is presented.

2. The symptoms were controlled or aggravated at will, by elimination or introduction of the offending allergen.

3. Improvement in personality, but not of the intelligence quotient, followed cessation of the cerebral edema.

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Special Article

A REVIEW OF CHEMICAL STUDIES ON THE ALLERGENS IN POLLENS

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THE substances which cause hay fever have not yet been isolated in pure form so their exact chemical nature remains obscure. New methods by which compounds of large molecular size can be analyzed and great improvements in technical apparatus have opened new fields for study. These methods are now being applied to the problems of pollen chemistry. A review of the extensive literature which describes the many previous studies of this subject and a discussion of the present state of our knowledge form the basis of this paper.

A large number of studies on pollen have been made by botanists, plant breeders, entomologists, and paleontologists but, since these studies throw no light on the chemical nature of allergens, they do not come into the field of this discussion.

EARLIER STUDIES

The earlier history of hay fever has been reviewed by many authors. Thommen[1] has traced the awakening of interest in the subject to as early as 1565, but recognition of it as a well-described disease was not forthcoming until 1819 when Bostock described the symptoms so well that for a long time what we call "hay fever" was known as "Bostock's catarrh." The idea that this catarrh might be due to pollens was first mentioned in 1831 when Elliotson recognized that the worst of the symptoms came with the maturing of the grasses in the haying season. Actual experiments, however, were not carried out for another thirty years. In 1873 Blackley[2] published a book in which he summarized the medical literature and described his own original work which had been carried on for fourteen years. Blackley studied the pollens from seventy-six identified species of plants and from several unidentified exotics. He found that the grasses could all produce hay fever in varying degrees. He used several ways of testing for potency. He applied decoctions to the conjunctiva, and he scratched them into the skin as in vaccination. It was Blackley who introduced the idea of comparing the intensity of hay fever symptoms with the amount of pollen in the

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air. In order to do this he devised a clocklike instrument which would cover and uncover slides carried high in the air by means of a kite.

The omission of chemical investigations of pollen was not due to any oversight, for Blackley persuaded a Mr. Thomas Harrison of Manchester to make a spectrographic examination of pollen. Sodium and barium were found. Blackley wrote as follows: "There is one part of the subject which would well repay careful investigation, viz., the chemical constitution of the pollen of various plants, and especially those of the order Graminaceae. Into this I have not been able to enter, but I cannot doubt that it would be well worth the time and trouble which would have to be expended on such an enquiry, if some able chemist would take up the matter and ascertain all that can be known of the constitution of pollen."

About this time the progress of hay fever studies was influenced by the spectacular successes in identifying bacteria as the cause of a number of diseases. The distinguished scientist Helmholtz, in 1869, proposed the theory that hay fever might be of bacterial origin.

Diphtheria was shown to be due to a toxin of bacterial origin. The successful immunization of animals against this material and the use of immune sera in the treatment of disease stimulated Dunbar[3] and his assistant, Kammann, in 1903, to study rye pollen on the assumption that the hay fever-producing substances in it were also toxins.

Dunbar supposed that he had found a cure for hay fever by means of the injection of serum from immunized animals. Interest in this idea continued for a time, but gradually the efficacy of the treatment was questioned and finally it was abandoned.

However, Kammann's[4, 5] investigation of the chemistry of rye pollen was thorough and became a model for later work. He made quantitative analyses for water, organic matter, and ash constituents and then studied the distribution of the organic material as ether soluble, alcohol soluble, carbohydrate, nonprotein nitrogenous material, and protein. The activity was found to be in this latter protein fraction as shown by scratch test, eye test, or intracutaneous injection in sensitive persons. Dunbar[3] and Kammann introduced the intracutaneous test in the study of allergy. Further study of the protein fraction was made by precipitating it with alcohol, redissolving, and reprecipitating it with saturated ammonium sulfate. The active material, freed from some impurities, remained in the filtrate. It was so soluble that it was not precipitated from solution by half saturated ammonium sulfate but only by a fully saturated solution. The material thus obtained was fairly stable on heating for one hour at 60° C. but it was destroyed to a greater extent at higher temperatures. It was not destroyed by 2.5 per cent sulfuric acid although it was easily destroyed by alkali. Also it was not destroyed by the proteolytic enzymes, pepsin and trypsin.

Kammann's work[6] continued for ten years during which he investigated such things as the enzymes in pollens, but his chief concern was a comparison of the substances causing hay fever with the toxic proteins isolated from other sources. Using Dunbar's method of extraction with 5 per cent sodium chloride solutions and precipitation of the extract by alcohol, followed by digestion of the starch with diastase, he obtained a white powder which was 100 times as active as the original pollen. This gave reactions characteristic of proteins such as the biuret test, though it did not give Millon's test for tyrosine, nor did it give Molisch's test for carbohydrates. Since a small amount of inactive material did not go back into solution, Kammann concluded that the globulins were inactive and that the active material was an albumin. It is interesting to note that he found,[7] by filtering mixtures of pollen toxin and horse antitoxin, that the pollen active substance was very much smaller than the antitoxin. Recent, more exact determinations of the relative molecular sizes have confirmed this.

Meanwhile the progress of research had continued. The discovery that animals which had been injected with a protein soon developed a sensitivity resulting in severe symptoms following a second injection of the same protein evidently influenced Wolff-Eisener,[8] who, in 1906, published a monograph on the history, diagnosis, and treatment of hay fever. In his concluding paragraph Wolff-Eisener discussed the possibility that hay fever might be a protein sensitivity. This conception was offered by him as a basis for further research.

In 1910, Meltzer[9] noticed the similarity of asthma to anaphylaxis and stated that, in the case of asthma associated with hay fever, it had been established experimentally that the sufferer is sensitive to the toxalbumin of pollen.

In 1911, the clinical study of hay fever as a protein sensitivity was further advanced when Noon[10] turned away from the idea of passively immunizing his patients by means of the sera of lower animals. He found that active desensitization of hay fever subjects could be obtained by the injection of water extracts of pollen. Noon attempted to get a mathematical basis for comparison of the extracts which he used and so he proposed that the unit of pollen be the quantity of pollen toxin which can be extracted from the thousandth part of a milligram (1 gamma) of pollen. He was using timothy at the time, and he thought that extracts of other pollens could be standardized in relation to this by comparative tests in the eyes of hay fever patients.

In 1914, Koessler[11] summarized the previous studies of pollens as a cause of hay fever in Forchheimer's text book of therapeutics. In discussing the nature of the active principle, he says that the alcoholic precipitate can be fractionated into pseudoglobulin, euglobulin, and albumin, the latter amounting to 16 per cent of the extractable protein

and containing all of the activity. Unless there were proteins present, there was no activity. The active material was found to be relatively thermostable and not appreciably weakened by heating to 70° C. for one hour. It was diminished but not destroyed by treatment with acids, alkalis, pepsin, or trypsin.

Thus, by 1914, the evidence which had been accumulated indicated that the active principle of pollen was a protein, so that Cooke,[12, 13] in following Noon's method of treatment of hay fever by injection of pollen extracts, compared his extracts on the basis of the total nitrogen which they contained.

Still the chemical composition of pollen had not been determined. This condition prompted Heyl[14] to start a series of quantitative and qualitative analyses of ragweed pollen. His first work was approximate analysis, dividing the extract up into such classifications as alcohol-soluble material, 42.9 per cent; moisture, 5.3 per cent; crude fiber, 12.2 per cent; pentosans, 7.3 per cent; ash, 5.4 per cent; dextrin, 2.1 per cent; and protein, 24.4 per cent. These figures represent an analytical scheme but do not tell us much about the chemical individuals present. By eye test on two persons it was determined that the alcoholic precipitate, which was described as a protein, was active. The separation by alcohol was evidently not very clear cut since one person also reacted somewhat to the supernatant liquid. Following this preliminary work, Heyl continued with a study of the proteins.[15] In the course of his experiments he used 1,150 Gm. of pollen which was defatted with ether and then extracted in succession with water, 10 per cent sodium chloride solution, and, finally, with weak alkali (0.2 per cent KOH; 0.2 per cent and 0.1 per cent NaOH). The water extract yielded 1.2 per cent of a substance coagulating at 45° to 50° C. in the presence of a small amount of acetic acid. This was called an albumin. Substances not coagulated by heat but precipitated by saturated zinc sulfate or ammonium sulfate were classified as proteose. The figures are hard to follow, but apparently this amounted to 3 per cent of the pollen. A mixture of this proteose and albumin sensitized guinea pigs so that, when reinjected later with the same material, they succumbed to delayed shock. After the removal of the proteins and proteose from the solution Heyl was able to identify qualitatively peptones, adenine, guanosine, histidine, arginine, and agmatine. From the material not soluble in water but soluble in the dilute alkalis, a glutelin was obtained amounting to 2.9 per cent of the pollen.

Further studies by Heyl[16] showed that the yellow coloring substances in pollen are flavonols such as are present in many flowers.

Later, analyses of the protein[17] were made to determine the distribution of amino acids. However the samples were not pure substances so that the most interesting findings of this part of the work are that

tryptophan, histidine, and cystine were not found in the albumin or proteose though the two latter amino acids did occur in the glutelin. Tyrosine was present in all in spite of the fact that Kammann had reported a negative Millon's test with his protein.

Heyl made a study of the sterols[18] and found a new phytosterol and also other higher alcohols. Further work on the alcohol and ether extracts[19] showed that; "the fatty acids in the ether extract include formic, acetic, valeric, lauric, and an unsaturated acid $C_{10}H_{18}O_2$, oleic, palmitic, and myristic acids,—the alcoholic extract contained a mixture of phosphatides—the water soluble part of the alcohol extract—yielded a trace of guanosine, adenine, betaine,—sucrose, and levulose. Glucosides were absent."

"The application of a systematic scheme of plant analysis to ragweed pollen has therefore yielded about 27 well defined substances, but with the exception of the coloring substances and possibly the proteose obtained from the water soluble fraction, none of these substances appear to represent any chemical specialization in this cell."

The work of Heyl has been presented rather extensively because the studies of this one man demonstrate well the complexity of the chemical constitution of pollen. Unfortunately Heyl's work did not do much to increase our knowledge of the active material since his attempts to test his materials for allergenic activity resulted in such serious reactions on the part of the subject that he ceased further experimentation by that method.

Similar, although less exhaustive, studies of the gross chemical constituents of pollen were made at the same time (1918) by Koessler,[20] who determined the amounts of alcohol and ether extractives and total nitrogen for the whole pollen of ragweed and the nitrogen distributed between coagulable and uncoagulable material extracted by 5 per cent sodium chloride. She also isolated as well as possible the amino acids of the proteins. Incidently she showed, by means of staining with Lugol's solution, that ragweed pollen, in contrast to rye pollen, does not contain starch.

ACTIVE NONPROTEIN SUBSTANCES

Numerous investigations had indicated that the active material in pollen was a protein, and this view was widely accepted in the early nineteen-twenties. Into this peaceful scene burst the announcement, in 1923, by Grove and Coca[21] that all of the nitrogen of a pollen extract can be eliminated by thorough dialysis without lessening the atopic activity. This statement has split the workers in the field into two camps and has led to many discussions.

It was at this time that Heidelberger and Avery[22] had isolated a soluble specific substance from pneumococcus and had shown it to be a

carbohydrate. This substance reacted with antisera in high dilutions but was, by itself, unable to stimulate antibody production when injected into animals. It was a haptene.

This work has had tremendous influence on the chemical studies of pollen. Since the active principle was possibly not a protein, many workers took up the idea that it might be similar to the specific carbohydrates. Black[23] assumed that the active material was not a protein since he could not obtain qualitative tests for proteins in the solutions which he used for treatment. That this argument is not sound was pointed out shortly by Alles, who called attention to the fact that various conditions modify the usual qualitative tests. Alles[24] concluded that actual isolation as a pure material and determination of molecular weight with analysis of the elements present was the only absolute criterion on this point. This stimulated Black[25] to isolate, in 1931, a substance of carbohydrate nature by precipitation of an aqueous extract of pollen with 1.4 volumes of alcohol followed by solution in physiologic saline and acetic acid at pH 4.4 and reprecipitation. The material thus obtained gave no tests for protein, though it contained 6 per cent nitrogen and 55 per cent reducing sugars after hydrolysis. It was specifically active when tested on the skin and nasal mucosa of ragweed sensitive patients.

The carbohydrate fraction of pollen was further studied by Caulfeild[26] who, together with Brown and Waters,[27] described its preparation. The method is rather drastic. It involves precipitation of the protein at pH 4.2 followed by clearing of the carbohydrate material with basic lead acetate, removal of the lead with cold dilute sulfuric acid followed by barium hydroxide, concentration under reduced pressure at 40° C., dialysis, and reprecipitation with several volumes of alcohol. The precipitate is taken up in 5 per cent trichloracetic acid, and then a final precipitate from alcohol is dried with alcohol and ether. The final product amounted to approximately 2 per cent of the pollen and, after hydrolysis, it yielded 58 per cent of a reducing sugar. It contained 1.5 per cent of nitrogen. It must be a very stable substance since it retained its activity, as shown by positive scratch tests on ragweed patients, and its ability to elicit a positive reaction following passive transfer. It could not cause anaphylactic shock in guinea pigs sensitized by injections of whole pollen, but it did inhibit reaction to the whole pollen extract in the Dale test. On the basis of these properties the carbohydrate was used in the treatment of pollen asthma. It was the impression of Caulfeild[28] that the asthmatic attacks were relieved more satisfactorily by this than by other methods of treatment.

Service,[29] in 1937, studied the question of pollen carbohydrates. He also was able to isolate polysaccharides from several western pollens through the use of lead acetate to remove proteins at pH 7 and to precipitate the active materials, as the solution was made alkaline with

ammonia. The lead was removed with hydrogen sulfide and the polysaccharides precipitated with alcohol. They could be redissolved in glacial acetic acid. These materials, in contrast to those of Black and of Caulfeild, did not produce skin reactions in man. However, anaphylaxis in the guinea pig could be inhibited by the administration of the polysaccharide previous to the shocking dose of antigen, and the Dale reaction could likewise be inhibited. In two cases it appeared that asthmatic attacks in humans were lessened by treatment with the polysaccharide.

Also in 1937, Harley,[30] in England, was working on a carbohydrate fraction from timothy pollen. The skin reactive potency of this fraction was slight as compared with the flocculated protein, and Harley considered that this might be due to the presence of small amounts of active albumin as a contaminant.

ACTIVE PROTEINS

The protein nature of the active material of pollen has had its strong supporters. Since the work of Grove and Coca, in 1923, many active preparations of proteinlike materials have been made. The chemists of the U. S. Department of Agriculture have made considerable study of the proteins of agricultural products, especially cottonseed. The allergic phase of this work started by Jones and Csonka[31] is still being carried on by Spies, Stevens, and others[32, 33] in collaboration with Bernton and is mentioned here since the methods used may have a bearing on the study of pollens. In 1925 Csonka, Bernton, and Jones[34] prepared several proteins from timothy and orchard grass pollens. Using 10 per cent sodium chloride to extract the crude pollen and then heating the solution to remove coagulable proteins, they obtained a proteose which was dialyzed for ten days in running water and was then evaporated before a fan and precipitated with alcohol. This material was easily soluble and was found to be active by skin test. The coagulable protein, designated as albumin, was active in about 50 per cent of the subjects. In another study Bernton, Jones, and Csonka[35] obtained four active proteins from timothy pollen as well as proteose and albumin from ragweed pollen. Autumnal hay fever patients exhibited a cutaneous sensitivity to either one or both of these.

Rappaport and Johnson[36] fractionated extracts of giant and low ragweed pollens with ammonium sulfate. The products were designated as: globulins, precipitating at 50 per cent saturation; a mixture of globulin and albumin, precipitating at 64 per cent; and albumins, precipitating at full saturation. These were tested for activity by diluting the extracts to 0.0025 per cent nitrogen and scratching this into the volar surface of the arm with a special instrument, making a 1 mm. cut not deep enough to produce bleeding. Their results are somewhat con-

fusing but the activity of the low ragweed proteins was greatest in the globulin fraction while that of the high ragweed was almost completely limited to the albumin.

It should be noted that the terms globulin, albumin, and proteose were originally used to designate classes of animal proteins, and there is danger in applying them too strictly to the materials found in pollens and other plant substances. Until such things as the molecular size and structure of pollen substances are determined more accurately, it is a waste of time to split hairs over their classification. This has been pointed out well by Moore and Moore[37] and more recently by Spies[32] and others. Even the serum proteins, which had been so nicely classified into euglobulin, pseudoglobulin, and albumin, are now, in the light of recent studies, found to be composed of large numbers of components.

In 1930 a series of studies was begun by Stull and others in Cooke's clinic.[38] These workers have been the most active proponents of the theory of the protein nature of the hay fever-producing substances in pollen.[39] Their earlier work[40] resulted in the isolation of an active protein. In one such preparation they found 13.52 per cent nitrogen, 50.37 per cent carbon, and 6.70 per cent hydrogen; these values agree well with values for the analysis of protein. The preparation was active in high dilution when tested by intracutaneous injection or by passive transfer. The authors considered this to be the only active substance in pollen, but more recently[41, 42] they have modified this view since they were able to separate two fractions of widely differing nitrogen contents. One of these contains 12 per cent nitrogen and is therefore similar to proteins while the other has less than 2 per cent and apparently also contains carbohydrate.

Active albuminous fractions were also obtained from low ragweed[43] and timothy pollens.[44] In contrast to these, cat-tail pollen, which is allergenically inactive, contained no protein.

Abramson, in collaboration with Sookne and Moyer,[45] has used the newly developed instruments of physical chemistry to study ragweed pollen. By studying the rate of movement of particles coated with ragweed substances in a cell through which a small electric current is flowing, they determined the isoelectric point as well as the titration curve of rate of movement as related to acidity. These values constituted evidence that the skin-reactive constituent in ragweed is protein in nature. One confusing point is that, during electrophoresis of ragweed extracts into the skin, reactions can be produced by either pole of the current,[46] but this may be explained by the fact that there is more than one active substance present.

The most significant feature in this study of Abramson and his coworkers[47-50] has been the isolation, by means of the Tiselius electrophoresis apparatus, of a colorless substance from both low and high rag-

weed pollen which is very active when tested by scratch tests. Several other constituents were found, but a complete study of their activity has not yet been reported.

MULTIPLE NATURE OF ACTIVE SUBSTANCES

A good explanation for the apparent discrepancy of the results of different workers in identifying the active material of pollen is that a number of different substances is involved. This has been suggested by many authors. Caulfeild[51] and his coworkers have consistently upheld this idea since 1925 when they found that tests with chemically different fractions of ragweed pollen resulted in varying degrees of reaction in different hay fever patients. Bernton and others showed that, for the pollens of timothy and orchard grass, the toxic action upon susceptible persons is not restricted to the albumin. Moore, Cromwell, and Moore[52] found that fractions from giant ragweed and timothy pollens, when tested by the method of exhaustion of locally sensitized sites, gave evidence of containing at least three distinct antigens. Johnson and Rappaport[53] found in high ragweed a globulin and an albumin antigenically distinct from each other. Their carbohydrate fraction was inert by skin test, and they concluded then that the active principle was of a protein nature or that it clings to a protein moiety. Hecht, Rappaport, and Welker[54] distinguished between what were presumably large and small proteinlike molecules from ragweed pollen, both of which were very active by scratch test. Stull[42] and also Abramson[50] have presented evidence of multiple substances connected with the skin reactivity of ragweed pollen.

ACTIVE SUBSTANCES SPECIFIC TO POLLEN

Pollen has been found to be the only part of the plant containing its special active substance. Walker[55] found that the leaves of trees have a different antigenicity than their pollens. Thommen[1] tested, by the intracutaneous method, extracts made from leaves, stems, roots, and seeds of high ragweed and timothy. The responses were invariably negative when the nitrogen content of the extracts was similar to that of pollen extracts giving positive reactions. Farmer[56] showed that there was no relationship between the pollen and seed antigens of grasses, and Manteufel and Wichelhausen[57] found that seed antigens from legumes could stimulate guinea pigs to produce antibodies sensitizing the human skin while pollen antigens did not have this potency. Bernstein and Kirsner[58] were able to get an atypical response consisting of induration and tenderness without wheal formation at the site of a passive transfer of ragweed sensitive serum when the recipient was fed powdered ragweed leaf. Apparently, in this instance, too, the leaves did not contain the pollen specific substance. Grubb and Vaughan[59] found

that only about one-half of the patients allergic to the pollen of food plants (wheat, corn, and beet) were also allergic to the corresponding foods.

ASSAY OF THE ACTIVITY OF POLLEN EXTRACTS

The methods of testing pollen extracts for hay fever-producing activity are still unsatisfactory, and this hampers quantitative studies of the chemistry of the active substances. Tests on the mucous membranes and scratch tests lack uniformity, and the results of intradermal injections are not quantitatively accurate whether read by the size of the reaction produced or by the greatest dilution of the extract which will produce a positive reaction. This has been discussed by many authors[40, 60] and is summed up in an article by Arbesman and Eagle.[61] Since the introduction of passive transfer by Prausnitz and Küstner,[62] this form of reaction has been used in a quantitative manner with fair success although it is a troublesome method and the selection of suitable recipients for the transfer is not easy. The substances causing the skin reaction and those neutralizing reagins may not be identical so that these different methods of testing for activity may not be strictly comparable.

The complement fixation test for pollen activity appeared very attractive and, in 1917, Clock[63] proposed using it. However Armstrong and Harrison[64] found that the complement-fixing property of ragweed pollen deteriorated rapidly and bore no relation to the substances which might cause shock when used for the treatment of hay fever. Therefore, for practical purposes, this test has been abandoned.

The weight of pollen used to prepare a given volume of extract was the early method proposed by Noon,[10] in 1911, to measure the strengths of extracts. He designated one unit as that quantity of the pollen toxin extracted from the thousandth part of a milligram of Phleum pollen. This method assumes that all samples of pollen are equally potent and, if used to compare pollen of different species, that all species have the same amount of active material. These premises are obviously wrong yet the Noon unit, because of its simplicity, is widely used in medical practice.

Clowes,[65] in 1913, using the eye test to follow the results of treatment, proposed that one unit be the amount of soluble constituents in one twenty millionth of a gram of pollen.

Koessler,[11] in 1914, limited the unit to protein and defined a unit as one hundredth part of 1 milligram of soluble pollen protein.

As noted previously, Cooke[12, 13] assumed that the active fraction was protein, and he standardized his extracts on the basis of their total nitrogen content. This method he, in collaboration with Stull, refined by isolating the protein nitrogen by precipitation with phosphotungstic acid.[66, 67] In this system one unit is one hundred thousandth (0.00001)

of a milligram of protein (phosphotungstic precipitable) nitrogen. Since this reagent will precipitate many things beside protein such as basic amino acids, urea, and even ammonium ions,[68] the Cooke unit is not a specific measure of protein.

Meanwhile, too, Coca[69] has reported that the precipitation by phosphotungstic acid may be prevented by inhibitory substances in some extracts, and he has shown that cat-tail extracts, which are allergically inert, give a large precipitate with phosphotungstic acid. Because of this, he wished to continue the standardization of allergenic extracts on the basis of total nitrogen and proposed a new definition of the Noon unit[70] as the quantity of pollen extract which contains one hundred thousandth (0.00001) of a milligram of total nitrogen.

Bowman's[60] studies of the skin reactions of old pollen extracts corroborated Coca's views.

Spain and Newell[71] found considerable phosphotungstic precipitable nitrogen in the inactive ultrafiltrates as well as in the active filter residues from ragweed pollen extracts. Sullivan and Vaughan[72] also found that the concentration of skin-reactive substance in extracts did not correspond with their protein nitrogen content.

In an attempt to make the protein nitrogen analyses more specific, Hubbard and Osgood[73-75] proposed that the precipitation of the protein be carried out by trichloracetic acid in 60 per cent methyl alcohol. This method would appear to yield better results than the others in such extracts as house dusts, as shown by Spain and Newell.[76]

The real trouble with all of the chemical methods of assay lies in the fact that there is no completely satisfactory knowledge of the chemical constitutions of the substances causing hay fever. Until this subject is cleared up, no chemical method can yield better than approximate results. On the other hand, tests for potency are limited so far to biologic methods which are difficult to interpret. The result of these conditions is that chemical studies of the active substances of pollens are not on a quantitative basis.

METHODS OF EXTRACTION AND PRESERVATION

The active material of pollen is soluble in distilled water and dilute aqueous solutions of salt or alcohol, and such solutions have been used for clinical and experimental purposes. The most widely used extracting fluids are usually modified physiologic saline solutions, either buffered or made slightly alkaline, plus some preservative such as phenol or high concentrations of glycerol or dextrose.

Water or saline extracts of pollen are quite acid. Stull[40] reports distilled water extracts of ragweed or timothy pollen as having a pH of 5.3 to 5.5. Johnson and Rappaport[53] found, for similar extracts a pH of 3.2, while Abramson, Sookne, and Moyer[45] obtained evidence of an isoelectric point between pH 3.9 and 4.3. Rockwell[76a] found that the

point of maximum precipitation of an unfractionated extract of low ragweed pollen was at pH 3.5 to 3.6, but in such a complicated mixture this is not necessarily the isoelectric point. Weak alkalis are, therefore, good solvents. Cooke[12] used 1/200 normal sodium hydroxide and Coca[77] recommended a solution of 0.275 per cent sodium bicarbonate and 0.5 per cent sodium chloride plus 0.4 per cent phenol in water as an extracting fluid for preparing extracts for clinical use. This solution is used by many laboratories and is very useful. Later, because the bicarbonate solution cannot be sterilized by heating, Grove and Coca[78] advocated the use of a buffered saline such as was suggested by Evans.[79] This solution, as used for preparing and diluting extracts, is made as follows: 3.63 Gm. of KH_2PO_4, 14.31 Gm. of $Na_2HPO_4 \cdot 12\,H_2O$, and 50.0 Gm. of NaCl are dissolved in water and made up to 1,000 c.c. Another solution is made containing 4 per cent phenol in water. For use, equal parts of these stock solutions are mixed and four times this total volume of distilled water is added. The phenol and salts are thus diluted to one-tenth of the original concentration. The resulting solution has a pH of 7.0 and can be sterilized by boiling or autoclaving. This should be done in a flask marked at the original volume so that the losses due to evaporation can be replaced, after cooling, by sterile distilled water. The pH should remain essentially unchanged.

Of course, once the extraction has been made, the solution should not be heated except for stable allergens such as glue and a few others. Sterilization is usually accomplished by filtering the extracts through bacteria retaining filters. Gelfand, Flamm, Center, and Heifitz[80, 81] found that the changes due to loss of carbon dioxide during vacuum filtration resulted in a loss of some of the nitrogenous material and produced a cloudiness, and therefore they recommended that filtration be done by applying pressure above the filter.

Saline extracts of pollen are stable enough for practical purposes but, over the course of weeks or months, the skin-reactive and reagin-neutralizing activity as well as the protein content of these solutions decrease. It is a matter of controversy as to whether these properties decrease at the same rate. This condition has prompted many workers to try to find another solvent with better preserving properties. Clock[63] introduced a fluid composed of two-thirds glycerol plus one-third saturated aqueous solution of sodium chloride. This he found by his complement fixation test to be better for extracting the ragweed antigen than glycerol or saturated salt alone and much better than distilled water. Stier and Hollister[82] found 46 per cent glycerol and 7 per cent sodium chloride in water to give the best combination of extracting and preserving properties, while Alles and Lamson[83] suggested the use of a phosphate buffer solution combined with the glycerine.

Glycerol is irritating when injected into the skin so Moore and Unger[84, 85] recommended 5 per cent dextrose and 0.1 per cent cresol in

distilled water for preparing pollen extracts. There is some objection to this solution in that molds are able to grow in it.

Strauss and Spain[86] recommended the use of sodium formaldehyde sulfoxylate (a compound of sodium sulfite and formaldehyde) to prevent oxidation of extracts of allergens. A larger amount of protein nitrogen was extracted by a solution of this from low ragweed pollen than was done by Coca's bicarbonate solution.

In 1915, alcohol was used by Goodale,[87] who added 13 to 15 per cent of alcohol by volume to his pollen extracts and reported that they apparently lost little efficiency over the course of one year.

The active material in dry form is very stable, and Rappaport[88] has shown that ragweed extract may be dried at 40° C. with very little loss in its solubility and no demonstrable change in its potency over eighteen months. This dried material may be used to prepare highly concentrated pollen extracts if desired, and it also provides a readily available supply for therapeutic purposes.

Stull and Sherman[89] have precipitated the protein of ragweed extracts with alcohol and ether at –10° C. This material is very stable in the dry state, and, when dissolved ten months later, it showed almost the same activity as fresh pollen extract. Meanwhile the original saline solution had lost protein and deteriorated considerably, as shown by its lessened ability to neutralize reagins in passive transfer tests.

A 3 per cent solution of ammonia was used by Baumann, Chudnoff, and Mackenzie[90] to extract the active substance from pollen. Such a solution was not intended for injection, however. The active fraction was precipitated by acetone and freed of ammonia by drying over sulfuric acid. These authors reported that three washings with the weak ammonia completely extracted the activity from pollen.

Solutions containing the condensation products of higher aliphatic acids and amino alkyl sulfonic acids[91] and the lauryl ester of sulfuric acid[92] have been patented for pollen extractants. It is interesting to note that some of these substances have also been reported as able to sensitize animals[93] so that their use in solutions for injection into human beings should be questioned.

The active substance is generally very difficult to remove completely from pollen grains. Murphy[94] found that five extractions with distilled water did not remove all of it. The dried residues were still capable of producing positive skin reactions.

Pollen is composed of small grains with a hard exterior[95] which might have a retarding influence on the extraction. Therefore, the earlier workers ground the pollen in a ball mill,[5, 29, 36] in a mortar with sand and glass,[12, 20, 63, 96] or broke it by repeated freezing and thawing.[10] Koessler[20] found that grinding ragweed pollen with sand for two hours did not increase the amount of nitrogen extracted. Langer and Kerr[97] have studied this question specifically, using ragweed and timothy pollens,

and have found that there is no qualitative and only slight quantitative differences between the extracts of whole and of ground pollen. They concluded that there is no practical advantage to be gained by grinding the pollen before extraction. Most workers have not attempted to break the pollen before extraction and have obtained good clinical results.[25, 77]

Fat solvents are generally used to wash the pollen before extraction for clinical use.[34, 40, 77] It is well known that the oils thus extracted may be the cause of dermatitis in some sensitive persons and that they are not involved in the production of hay fever. The skin test active material is practically insoluble in ethyl ether, petroleum ether, chloroform, benzene, and absolute alcohol.[38] Milford[98] found the oil extracted from pollen by fat solvents to be active only on some patients, while Moore, Cromwell, and Moore[99] investigated this and concluded that its skin-reactive substances are identical with one or more of the antigens extracted by aqueous solvents. Defatting the pollen has been shown to have no effect on the antigenicity of the extracts in rabbits.[100] The loss of the small amount of activity due to the ether washing is compensated by the easier extraction of the remainder by aqueous solutions and the clarity of the final extracts.[97]

PRECIPITATION, FRACTIONATION, AND INACTIVATION OF POLLEN EXTRACTS

What reagents may be used to precipitate the active materials from extracts? Are these reagents likely to destroy the activity? These questions would be much easier to answer if there were some reliable quantitative method for determining pollen activity. As it is, so many workers have used unrelated tests that only the most elementary comparisons may be made. The problem is further complicated by the probability that the activity is shared by a number of different substances.

Aqueous extracts are fairly stable, depending on their concentration. The active material may be precipitated from these by adding neutral salts in large quantities. There is no good agreement as to where the salting out begins. The activity is not precipitated by 5 volumes of saturated magnesium sulfate[4] nor by saturated sodium chloride.[63] Some workers have found activity in what is frequently called the globulin fraction, that is, the precipitate at half saturation with ammonium sulfate,[36, 42, 90] while others found that the activity was not thrown down until the solution was almost completely saturated.[4] These differences depend, most likely, on such things as the acidity of the solution or the concentration of the active material.

By changing the acidity of the solution to pH 4.2 with acetic acid the proteins of ragweed pollen extracts were precipitated in Caulfeild's[27] preparation of the carbohydrate although on the other hand, Stull[42] obtained proteins from a solution which had been previously brought

to pH 4.0. Hecht, Rappaport, and Welker[54] found that the material precipitated from ragweed extracts at pH 4 was the antigen which reacted with rabbit antiserum.

Different methods of preparing the active material yield products which are not alike in their precipitability by protein precipitants. Black's[25] carbohydrate gave no precipitate with pieric, tannic, or phosphotungstic acid nor with neutral lead acetate, or sodium tungstate, whereas Caulfeild[27] and Service[29] precipitated their carbohydrates with basic lead acetate. Stull's[40] protein gave precipitates with Millon's reagent, phosphotungstic, and tannic acids. Trichloracetic acid in water solution does not produce a precipitate in pollen extracts, but its use in the presence of 60 per cent methyl alcohol has been suggested as a precipitant in the chemical assay of allergens.[73] Except for basic lead acetate, none of these reagents have been used in the isolation of the active material, presumably because they destroy the activity. However Naterman[100a] made a precipitate in unfractionated ragweed extracts by adding 1 per cent tannic acid, and he used this precipitate for therapeutic injection with satisfactory clinical results.

Alcohol is a precipitant of the active material in pollen. Fifteen per cent alcohol dissolves it,[87] but many observers have found that about 70 to 80 per cent alcohol precipitates it more or less completely.[4, 25, 30, 40] Absolute and 95 per cent alcohol have been used for washing the pollen before extraction to remove a considerable amount of inactive material. However, alcohol at room temperature and high concentration is likely to denature proteins,[101] and therefore it should be avoided if possible, although Stull and Sherman used alcohol at –10° C. with satisfactory results.

Acetone[65] has been used as a precipitant of the active material, but it has the same disadvantages as alcohol.

Other organic solvents such as dioxane and isopropanol have been used to precipitate fractionally the active material in pollen by Boatner, Efron, and co-workers.[102, 103]

Concentrated phenol extracts a skin reacting substance from pollen, and it was used to sterilize whole pollen for injection by Murphy.[94]

Concentrated urea solutions were tried as solvents for antigens by Mackay and Lamson,[104] but the results were not very satisfactory. Urea solutions are known to cause the splitting of protein molecules into smaller ones,[105] and therefore the solutions might be expected to be unstable.

Rockwell[106] reported that after treatment with hydrogen peroxide much larger doses of ragweed pollen could be given to patients without causing reactions.

A patent[107] has been secured for the use of formaldehyde or methenamine for treatment of pollen solutions in order to "detoxify" them.

Stull and others[108] have studied pollen extracts modified by formolization, radiation with ultraviolet light, heating, acetylation, and alum precipitation and found that the clinical results were best in the group of hay fever patients treated with fresh extracts.

The agents which destroy pollen activity are heat and alkalis. Kammann[4] reported long ago that the activity was fairly heat stable, resistant to acids, but destroyed by alkali, and Noon[10] sterilized his extracts by boiling them for ten minutes in sealed tubes. Bouillenne and Bouillenne[109] found pollen extracts to be but slowly inactivated by heat. Gay[110] studied this and found that the activity, as measured by intradermal tests, is markedly reduced by heating to 100° C. for one minute or to lower temperatures for longer times at pH 7.4. Dilute extracts were inactivated much more rapidly than concentrated ones. Alteration of the hydrogen-ion concentration to pH 4.0 and to pH 8.4 in no way changed the effect of heat on the intradermal reaction produced by the extracts. He measured the potency by the size of the resulting wheal which is not a very exact method. Arbesman and Eagle,[111] using the reagin-neutralizing ability of ragweed pollen extracts as an index, found these to be highly thermolabile. Heating also appears to alter the antigenic specificity of pollens.[108] Thus, in spite of the earlier reports, it is evident that heat is definitely deleterious to pollen extracts.

Ragweed extracts are inactivated by adsorption on quartz particles, and this fact was used by Abramson, Sookne, and Moyer[45] as evidence of their protein nature.

Other substances absorb pollen active materials. Kammann[6] adsorbed it on fibrin and recovered it by elution with weak soda. He also adsorbed it on aluminum hydroxide, iron hydroxide, bone charcoal, kieselguhr, and yeast. Moore and Moore[112] investigated the efficiency of adsorbents in removing solids and nitrogen from giant ragweed and timothy pollen extracts. The activity of the extracts and filtrates was tested by direct skin test and by passive transfer on human beings and by skin test on sensitized rabbits. The order of efficiency of adsorption, as measured by nitrogen was the same as that measured by the skin-reacting activity in humans and rabbits. Active charcoal was the most active adsorbent, followed by iron oxide and alumina gel. Diatomaceous silica and silica gel, contrary to the results of Abramson,[45] appeared to cause no decrease in the activity of the extracts. Stull, Chobot, and Cooke[38] found that small amounts of norite (active charcoal), animal charcoal, kieselguhr, and alumina gel could remove about one-half of the nitrogen of pollen extracts without markedly diminishing their skin activity. Klewitz and Wigand[113] found the allergens of meadow grass to be absorbed and inactivated by animal charcoal but not completely adsorbed on kaolin or aluminum hydroxide.

Spain and Strauss[113a] used gelatin in pollen extracts to slow up their absorption following injection. However, this does not prove that the active material is actually adsorbed to the gelatin.

Westphal[114] has attempted without great success to fractionate pollen extracts by passing them through tubes packed with alumina, using the chromatographic fractionation technique.

ENZYMATIC DIGESTION

An attempt to throw light on the chemical nature of pollen activity by enzymatic digestion has been made by numerous workers starting with Kammann.[4, 6] He reported the active principle of rye pollen to be resistant to pepsin and trypsin. He used diastase to dissolve starch from pollen extracts without affecting the specific activity. Grove and Coca[21] thought that digestion with trypsin and dialysis would remove all the nitrogen from pollen extracts without lessening their activity. They maintained, therefore, that the active material is not protein. Stull,[39, 66] on the other hand, has collected a number of references to the enzymatic digestion of pollen extracts in support of the protein theory. He cites the studies of Melli,[115] of Farmer,[116] and of Bouillenne and Bouillenne[109, 117] who were able to inactivate grass and ragweed pollens by digestions with proteolytic enzymes. Benjamins, Dishoeck, and German[118] found pepsin and trypsin to diminish but not destroy the skin activity of pollen extracts, while Bernstein and Kirsner,[58] in connection with an investigation of oral therapy with ragweed pollen, found that digestion with gastric juice does not destroy the activity as determined by the scratch test. Rockwell[76a] arrived at similar conclusions from a study of pollen extracted by pepsin in hydrochloric acid. Moore and Unger[119] found the pollen allergen to be very slowly inactivated by proteolytic enzymes, and a similar finding was reported by Thiberge.[120] To sum up all these investigations, it would appear that the active principles of pollen are rather resistant to digestion by proteolytic[121] enzymes in the same way as are other plant proteins such as urease and some of the plant toxins.

ENZYMES PRESENT IN POLLENS

The enzymes of pollen have been studied by many investigators from various viewpoints. The botanical literature has many references to this subject, only a few of which will be mentioned here. From the allergist's point of view, Kammann was the first to study enzymes in hay fever-producing pollens. He found enzymes which digest fibrin, gelatin, and starch as well as catalase and lipase in rye pollen.[6] Paton[122] examined a large number of pollens and found amylase, invertase, catalase, and pectinase in all and zymase, erepsin, trypsin, pepsin, lipase, and reductase in some.

Grove and Coca[78] confirmed the presence of enzymes in pollen which digest gelatin and raised the question as to whether the deterioration in activity of pollen extracts can depend on these enzymes. This matter has not been settled although the digestion and loss of activity is a relatively slow process under the usual conditions. These enzymes can be inactivated by formolization, heating, acetylation with ketene, or radiation with ultraviolet light,[104] but these processes also reduce the serum-neutralizing potency of the extracts so they could not be used as practical measures for protecting the activity of the extracts.

MOLECULAR SIZE OF THE ACTIVE SUBSTANCES

The early workers attributed pollen activity to proteins or at least proteoses. They did not have any dependable method of measuring large molecular weights, and they used dialysis to separate large from small particles. Grove and Coca[78] reported that the pollen activity of ragweed did not dialyze to any great extent through collodion sacs, and this observation was supported by many other workers. On the other hand, Klewitz and Wigand[113] reported that meadow grass activity passed through membranes which would retain Congo red; therefore, they concluded that it was a small molecule. Farmer[116] later pointed out the need of knowing the pore size of the dialyzing membrane before using dialysis to determine whether or not a substance is protein. Unger, Cromwell, and Moore[123] found that pollen substances capable of producing positive scratch reactions in certain sensitive persons were dialyzable through gold-beater's skin, cellophane, and collodion. Johnson and Rappaport[53] reported the loss of considerable active material of protein nature from ragweed pollen extracts during dialysis through cellophane and collodion sacs. Spain and Newell,[71] using filtration under pressure through various grades of cellophane, found that the active principle of ragweed was only partially retained by the thin (No. 300) cellophane membranes but the very thick grade (No. 1200) retained almost all of the activity while allowing all of the nonprotein nitrogenous material and about one-third of the phosphotungstic precipitable nitrogen to filter through. Contrary to this Benjamins[118] and co-workers reported that, after filtering pollen extracts through an "ultrafeinfilter," they obtained protein-free solutions which gave no precipitates with phosphotungstic acid and were not completely inactivated when digested with pepsin and trypsin. The resulting solutions were inactive by skin test but, when mixed with a number of other substances ranging from glycerol and glucose to proteins of various origins, the skin reactions were considerably enhanced by the substances of large molecular size. These authors, therefore, suppose that the pollen substance is a small molecule which is activated by coupling with nonspecific colloids of which the proteins are most suitable. This work was repeated in a similar manner by Long and Teller,[124] using cellophane as

a filter. The filtrates of ragweed extracts were very much less active than the original extracts when tested by intracutaneous injection. Mixtures of the filtrates and egg albumen or casein produced slightly larger reactions than did either alone.

Hecht, Rappaport, and Welker[54] separated ragweed extracts into fractions by precipitation at pH 4 followed by heating to 60° C. and filtering. The precipitates, after being redissolved in weak alkali, were relatively easily precipitated and quantitatively adsorbed on alumina. They diffused very little through collodion and formed viscous solutions. These precipitates were thought to be composed of molecules larger than those in the filtrates. The evidence is not conclusive, but the authors claim that these results tend to confirm the work of Benjamins[118] and others.

Recently more reliable and direct evidence of the size of the active molecule has been secured by Abramson, Moore, and Gettner,[50] who used the ultracentrifuge and calculated the molecular weight to be about 5,000. Sanigar[125] also attempted to estimate the size of particles in unpurified extracts and found a marked heterogeneity with the greatest amount being composed of those having a molecular weight of less than 17,000. Most proteins have molecular weights of 35,000 or some multiple thereof so that from these experiments it is evident that the active substances in ragweed pollen are small as compared with the usual proteins.

A very interesting experiment in this connection is that reported by Winkenwerder, Buell, and Howard.[126] These workers found that persons sensitive to ragweed would give skin reactions to a number of simple chemical compounds derived from nucleic acids. Even urea gave typical reactions on some of these persons while control tests on normal persons or those sensitive to danders, dust, or foods were negative. Skin-sensitizing antibodies were demonstrated in the serum of ten persons reacting to these compounds. If this rather broad reaction on the part of ragweed-sensitive persons can be found by others, it will be very difficult to fit in with our usual conception of specific reactions.

ANTIGENIC PROPERTIES OF POLLEN EXTRACTS IN ANIMALS

The antigenicity of pollen extracts is so tied to the chemistry that it is well to consider this aspect of the subject briefly. It is assumed generally that particles of large size are necessary to stimulate the formation of circulating antibodies. Pollen extracts are on the border line of possessing this power. Experiments on animals show them to be slightly antigenic under the proper conditions. Koessler[11] reported that he could sensitize guinea pigs to anaphylactic shock with pollen protein. Goodale[87] was able to obtain precipitins to different pollens by injecting rabbits with them. Heyl[15] found some evidence of anaphylactic prop-

erties in ragweed proteins. Parker[127] sensitized guinea pigs with the same ragweed extracts as used in clinical treatment and demonstrated their sensitivity by the Dale test. This was confirmed by Walzer and Grove[128] although they could not obtain anaphylactic shock in guinea pigs nor obtain precipitins so concluded that pollens are not precipitinogenic. Alexander,[129] on the other hand, found that guinea pigs were sensitized and shocked by rye pollen extracts although he could not find precipitins or complement fixing antibodies in their sera. Harrison and Armstrong[130] obtained similar results with glycerinated extracts of ragweed pollen. By sensitizing rabbits or guinea pigs to another antigen such as horse serum at the same time as ragweed, Parker[131] was able to obtain precipitins; Bernstein[132] used the same method to obtain similar results with other pollens. Burky[133] used staphylococcus toxin to aid in sensitizing rabbits to ragweed. Armstrong and Harrison[134] obtained complement fixing antibodies by injecting rabbits many times with ragweed pollen extracts, and, similarly, Huber and Koessler[135] were able to produce fatal anaphylactic shock in guinea pigs and specific antibodies in rabbits by multiple injections of plain pollen extracts. Ramsdell[136] injected whole pollen into guinea pigs, which had been previously sensitized with a mixture of ragweed extract and horse serum, and obtained anaphylactic shock. Caulfeild, Cohen, and Eadie[137] produced anaphylactic shock in guinea pigs with their "proteose" fractions of ragweed, timothy, and birch pollens. Adelsberger[138] was not so successful and obtained only one case of shock in twelve guinea pigs sensitized to timothy pollen. Service[29] reported that the polysaccharides of pollens would inhibit the anaphylactic or Dale reactions of guinea pigs if they were administered prior to the pollen antigen. Skin reactions in sensitized rabbits were demonstrated by Cromwell and Moore[139] by the intravenous injection of trypan blue following cutaneous injections of ragweed or timothy pollen extracts. The same workers also found precipitins to be present[140] as did Hecht, Rappaport, and Welker.[54] Eagle, Arbesman, and Winkenwerder[141] regularly caused the formation of precipitins and complement fixing antibodies in rabbits by injecting ground pollen or saline extracts of low ragweed pollen. They also sensitized guinea pigs to anaphylactic shock with ragweed extracts.

In spite of the large number of successful sensitizations of animals with pollen extracts, the effect is not so easy to produce as when good antigens, such as serums or egg albumen, are used. With the introduction of the alum treatment of diphtheria toxoid, Harrison[142] investigated this method as a possible aid to therapeutic injections and found alum-precipitated ragweed pollen extract to be an effective agent in sensitizing guinea pigs to anaphylactic shock. This has been confirmed by Zoss, Koch, and Hirose[143] as well as by Coulson and Stevens.[144] Caulfeild, Brown, and Waters[145] found it difficult to sensitize guinea pigs with

plain pollen extract or extract plus staphylococcus toxin, but they obtained a high percentage of sensitizations with extract to which alum had been added.

The precipitation of pollen extracts by alcohol has an action apparently analogous to that of alum in rendering the activity less soluble in the body and therefore increasing its ability to stimulate the formation of antibodies. Manteufel and Kühne[146, 147] succeeded in producing anaphylactic sensitization of guinea pigs by injecting them with such a precipitate.

SUMMARY

The chemistry of the hay fever producing substances in pollens has been studied for about forty years. The sum of all this work is difficult to evaluate because of contradictory reports and lack of uniform methods for quantitative studies. However, there are a few facts. The activity is shared probably by several substances all of which are of complex chemical natures. The molecules are smaller than those of the true proteins, and one of the substances has been found to have a molecular weight of about 5,000. The rather low antigenic potency of pollen extracts and its enhancement by various adsorbents or other antigens is probably a function of this small size. Some of the active substances contain much carbohydrate, and others resemble proteins. They are inactivated by heat and alkaline media. They are slowly inactivated in the presence of proteolytic enzymes. However, they do not fit into the accepted classification of proteins though, for some, the term "naturally occurring proteoses" would seem suitable. As yet, none of the active substances have been isolated in pure form in large enough quantities to permit a thorough chemical examination so that their constitution is still not known exactly.

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Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND
THEODORE B. BERNSTEIN, M.D., CHICAGO

Occupational Asthma and Vasomotor Rhinitis. An Outline of Some Common Industries Where These Symptoms Are Initiated. Sternberg, L., and Sorrell, A. H.: New York State J. Med. 41: 1649, 1941.

The authors found the workers in the following industries most commonly affected with vasomotor rhinitis or asthma: bakers, beauticians, furriers, metal polishers, coffee, soy, cocoa, and castor bean handlers, refrigerator workers, jewelers, hat workers, rag sorters, woodworkers, poultry dealers, pharmacists, and upholsterers. Positive skin reactions usually are obtained in these industrial allergies when the causative agent is not a chemical. The offending agents in the case of bakers are generally cereal flours. Furriers may become sensitive to the fur itself, the dye (paraphenylenediamine or ursol D), an insecticide, such as paradichlorbenzine, generally used in the fur trade, or "chimoidiimin" (an incomplete oxidation product resulting from the reaction of ursol and hydrogen peroxide), which is used in the final stages of the dyeing process. Beauticians have symptoms from sensitization to orris root, hair dyes, and hair-waving solutions containing quince seed, flax seed, karaya gum, or gum tragacanth. The ideal treatment in most of these cases of industrial allergy is the removal of the offending causes.

Adrenaline-Ascorbate Suspensions in Bronchial Asthma. Kennedy, A.: Lancet 2: 279, 1941.

The author has prepared an adrenaline preparation with the object of delaying its absorption after injection, so that a long-continued and regular adrenaline action may be secured. The preparation consists of a concentrated suspension of dry adrenaline base with ascorbic acid in arachis oil, anhydrous wool fat, and a small quantity of white wax. The presence of ascorbic acid discourages oxidation of the adrenaline. This suspension contains 10 mg. of adrenaline base per cubic centimeter, and the average dose is 0.3 c.c., although it is preferable to use an initial dose of 0.1 c.c. In status asthmaticus when adrenaline is usually well tolerated the initial dose may be 0.5 c.c. The suspension has proved particularly useful in status asthmaticus and in chronic asthma with frequent nocturnal attacks.

Studies in Atmospheric Pollen. Phillips, Marie E.: M. J. Australia 2: 189, 1941.

A pollen survey has been conducted in New South Wales, and the present article is a preliminary report of the data obtained from eleven stations over the period September 1 to December 31, 1940. During this period regular counts indicate two distinct pollen seasons: (1) the spring or tree season and (2) the summer or grass and plantain season. Weather factors, such as rainfall, sunshine, tempera-

ture, wind direction and velocity, and humidity, were studied and correlated with the counts. The effects of rain, sunshine, and temperature were clear cut and coincided with previously reported studies. Those of wind and humidity were less obvious. The instrument used for collecting the pollen was a movable weather vane type with horizontal and vertical slides. The author prefers this method to the single horizontal slide.

Dermatology

UNDER THE DIRECTION OF MARION SULZBERGER, M.D., NEW YORK, AND JOSEPH GOODMAN, M.D., BOSTON

The Poison Ivy Plant and Its Oleoresin. Shelmire, B.: J. Invest. Dermat. **4**: 337, 1941.

This paper presents several major contributions to our knowledge of poison ivy and the dermatitis produced by this plant. The author demonstrated experimentally that the three species of poison ivy are in all likelihood a single pleomorphic species. At any rate, the saps and the ether-extracted oleoresins of the three species of poison ivy and of poison sumac have been demonstrated to be identical from an immunologic standpoint. It was also demonstrated that the potency of the oleoresin derived from various plants of the same species showed a marked variation. Likewise the leaves gathered at different seasons from a single plant presented differences in potency as judged by the extracted oleoresin.

The author further demonstrated that the extracted oleoresins deteriorated very slowly, if at all, in the presence of air or in the presence of tap water. The potency of the oleoresin was lessened by boiling both green and dried plants for twelve hours in water.

Studies in Contact-Dermatitis. IV. The Spontaneous Flare-Up of Negative Patch Test-Sites in Experimental Sensitization in Man. Grolnick, M.: J. Immunol. **41**: 127, 1941.

The author presents a study of the flare-up of patch test sites after patch tests in *Krameria*. The spontaneous flare-up of the site of an initial sensitizing application occurred in eighteen subjects with an incubation period of eight to twenty-one days. In nineteen subjects in whom two to five applications were necessary to produce sensitivity there was a spontaneous flare of the site of the application immediately preceding that which sensitized. In a few instances the sites of two preceding applications presented spontaneous flares. The author terms this period between the time of application and the time of flare the *inactive phase*. It is to be noted that this term is used only in connection with a flare-up which occurs when sensitivity has been induced by more than one application of the excitant. The sites of previous tests remained inactive for ten to forty-three days prior to their flare-up. The flare-up at sites always occurred in the reverse order of application.

False Positive Serologic Reactions for Syphilis in Infectious Mononucleosis. Kaufman, R. E.: J. Lab. & Clin. Med. **26**: 1439, 1941.

Among eighty-two proved cases of infectious mononucleosis there were three patients with positive serology. Of the eighty-two persons, sixty-four had one serologic test for syphilis during the acute stage of the illness and thirty-two had tests on two or more occasions. In all three patients who presented positive tests

there was a reversion to negative within ten weeks. The test which showed a positive reaction was not always the test which is ordinarily considered the most sensitive. There was no correlation between the sheep cell agglutination titer and the serologic tests.

A review of the literature indicates that between 8 and 15 per cent of patients with acute infectious mononucleosis have been reported to present positive serologic tests. The author's experience, as well as unpublished observations of other observers, indicates that the actual incidence may be between 2 and 10 per cent.

Studies in Bite Desensitization. McIvor, B. C., and Cherney, L. S.: Am. J. Trop. Med. **21**: 493, 1941.

Encouraging results were obtained in attempted desensitization of sixty-seven patients who manifested a hypersensitivity to the bites of fleas. The antigen used was prepared from the dog flea and the human flea. Extraction of the powdered fleas was made in a dilution of 1:100. A 1:5,000 dilution of the extract was used for injecting. A course of injections for an adult consisted of six injections; the first dose was 0.2 c.c. and subsequent ones, 0.4 c.c. of the diluted extract, injected intradermally or subcutaneously. After desensitization the wheal produced by the bite of the insect was smaller in size; its duration was shorter; and irritation and pruritus were decreased. In addition it is reported that the majority of the patients so treated no longer found fleas on their persons or in their clothing. With one exception (a ground squirrel) long-continued contact with fleas or desensitization with flea extracts did not inhibit the culture of various fleas on various experimental animals.

Present Status of Tuberculin Patch Test. Kereszturi, C.: Am. Rev. Tubere. **44**: 94, 1941.

The author reviewed the nineteen reports of the comparison of the tuberculin patch test with other standardized tuberculin tests. For statistical analysis he selected nine reports of comparison with the Mantoux test. In a total of over 4,000 tests there were 270 instances in which the Mantoux test was positive while the patch test was negative. There were also seventy-eight instances in which the patch test was positive while the control test was negative.

The author's study of 379 children revealed thirty-one negative patch tests in Mantoux-positive patients and twenty-eight positive patch tests in Mantoux-negative children. Further study revealed that the discrepancies between the two tests occurred for the most part in patients with a low degree of tuberculin sensitivity. The author concludes that the tuberculin patch test, because of simplicity of performance, has a limited field of usefulness but that it is not as yet as reliable as the well-established Mantoux test.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., SAN FRANCISCO

Intradermal Immunization. Scarlet Fever. Fischer, S., and van Gelder, D. W.: Am. J. Dis. Child. **61**: 88, 1941.

The Dick test was given to 1,120 institution children between 1.5 and 19 years of age. A total of 139 showed positive reactions. Of these, 120 were then given

three injections of scarlatinal streptococcus toxin. Half received 800, 1,600, and 3,200 skin test doses, respectively, and half received 1,600, 3,200, and 8,000 skin test doses. In all a reversal of the Dick test was obtained when tests were made three months later. Fifty per cent of the children receiving the larger dose had some constitutional complaint following at least one injection. This consisted of fever, vomiting, headache, or rash in order of frequency. Urinalyses were negative.

Of the children receiving the smaller dose of toxin, 21 per cent had a constitutional reaction after at least one injection, and again urinalyses were negative. There was no relationship between severity of reaction and age which is contrary to most reports. The intradermal method of immunization against scarlet fever is felt to be superior to the subcutaneous method because three rather than five injections are given and reactions are fewer and milder.

An interesting observation in the course of the study was that in the instances when it was closely followed almost half of the children developed a positive reaction to the control test after immunization indicating a hypersensitivity to the protein of the toxin. This tended to reach its height three months after the last dose and then to disappear gradually.

Intradermal Immunization. Diphtheria. Blatt, M. L., Fischer, S., and van Gelder, D. W.: Am. J. Dis. Child. **62**: 757, 1941.

Of 945 children between 1 day and 19 years of age who were given Schick tests, 228 reacted positively. They were immunized intradermally with either alum-precipitated toxoid or plain toxoid, both of which seemed satisfactory as immunizing agents, according to the authors. Due possibly to the small number in each group, however, there was considerable variability of response. For example, in thirty-eight children given two doses of 0.1 c.c. and 0.2 c.c., respectively, of alum toxoid, there were none with positive Schick tests at the end of eleven months. Of twenty children who were given a larger total dose (two doses of 0.2 c.c. each), three had a positive Schick test nine months later.

Systemic reactions were minimal even in the older age group, and it is this fact which recommends the intradermal method. Unmodified toxoid produced less severe local reactions than alum-precipitated toxoid, which may cause considerable immediate pain on injection, lasting several hours. Alum toxoid occasionally caused sterile abscesses as well. The preferred method of diphtheria immunization is stated to be the intradermal injection of two doses of 0.1 c.c. and 0.2 c.c., respectively, of unmodified toxoid at an interval of three weeks, followed by a third if the Schick reaction is still positive.

Intradermal Immunization. Typhoid Fever. Van Gelder, D. W., and Fischer, S.: Am. J. Dis. Child. **62**: 933, 1941.

Five hundred and eighty children in the Illinois Soldiers' and Sailors' School between 1.5 and 19 years of age were immunized against typhoid fever. Fifty per cent were immunized intradermally, using a total dose of 0.25 c.c. to 0.45 c.c., and others were immunized subcutaneously with a total dose of 2.5 c.c. Local and general reactions were very much fewer in the intradermally immunized group. Their H, or flagellar, agglutinin titer was as high as that of the subcutaneously immunized group, but their O, or somatic, agglutinin titer was much less. Whether an interval of two or three weeks was used did not affect the subsequent agglutinin response. A total dose of 0.30 c.c. was recommended when the intradermal method was used in subjects weighing 120 pounds or more.

Amino Acids as a Source of Nitrogen for Allergic Infants. Hill, L. W.: J. A. M. A. **116**: 2135, 1941.

A synthetic protein-free food, prepared by casein hydrolysis and intended for use as a milk substitute, was fed to thirty-six infants for from two weeks to three months. It appeared to be as satisfactory a milk substitute clinically as certain other preparations commonly used. It was thought to be especially suited to very young infants. Its chief disadvantage was its taste, which resembled strong beef tea, and caused some infants to refuse it either from the start, or after a short period of time.

Specifically, satisfactory results were obtained with this milk substitute in half the cases, while in a quarter they were unsatisfactory, and the remainder inconclusive. The adequacy of the preparation as regards nitrogen metabolism for short periods at least, had already been demonstrated.

It is pointed out that no milk-free diet for infants is without disadvantages and that it is desirable to have several types available.

Active Immunization Against Tetanus and Diphtheria in Infants and Children. Bigler, J. A., and Werner, M.: J. A. M. A. **116**: 2355, 1941.

The unsatisfactory status of tetanus prevention by means of antitoxin induces interest in a possible alternate solution by active immunization with toxoid. Since 50 per cent of tetanus deaths are in children, their protection is particularly important. Two hundred and forty infants and children from 8 months of age to 6 years of age were included in this study, the body of which concerns itself with alum-precipitated combined diphtheria and tetanus toxoids. In most instances two injections were given three months apart, and a third was given one to twelve months later. A shorter interval between the initial injections is considered less desirable. Either 0.5 or 1.0 c.c. of toxoid was given. The response, as measured weeks and months later in tetanus and diphtheria antitoxin units, was good. Granting we do not know exactly what level of tetanus antitoxin is protective, it was apparent that higher levels are found one week after the third and even after the second injection than are obtained with the usual prophylactic antitoxin injections. These levels were also maintained for weeks rather than for a few days. In one child there was a failure of both diphtheria and tetanus response. Reactions were few. No abscesses occurred. Urticaria was encountered twice; in one case, a few hours after injection; in the other case, immediately after injection. The general febrile reactions were fewer and milder than occur with pertussis vaccine. Approximately one-half of the group were allergic persons.

It is concluded that it would seem safe to give previously immunized persons a stimulating dose of tetanus toxoid after an injury involving probable infection with tetanus spores.

Sensitivity to Horse Serum Due to Previous Injections of Antigen. Toomey, J. A., and Garner, W. P. Am. J. Dis. Child. **62**: 765, 1941.

Fourteen hundred patients of whom 207 had diphtheria, seventy-five had meningitis, sixty-one had erysipelas, and 1,057 had scarlet fever are included in this statistical survey. These patients were admitted to the City Hospital of Cleveland between 1934 and 1938. All were given intradermal tests of 0.1 c.c. of 1:10 dilution of horse serum and also conjunctival tests with the same material. Irrespective of the outcome all but two then received specific therapeutic serum. The two in whom it was omitted had a general reaction following the tests.

Of the 1,400 patients, 448 had a history of previous administration of diphtheria toxin-antitoxin. Forty-four had had horse serum in some other form, and 908 had not had previous injections containing horse serum.

Those who had previously had horse serum gave a higher percentage of positive tests (22 per cent as compared with 16 per cent), but they were not more likely to have serum sickness.

If the intradermal test was positive, there was a slightly greater likelihood of serum sickness than if it was negative. If the conjunctival test was positive, as it was in 3 per cent of the entire group, there was a 48 per cent likelihood of the occurrence of serum sickness.

Practically, these data meant that, if a patient had a positive cutaneous test to horse serum, it would be of no value to know whether or not he had had serum previously when predicting whether serum sickness would develop.

The results did not confirm the view that administration of serum sensitized human beings if serum sickness is used as the index of such sensitivity. Using the skin test as the index, however, there was an increase in sensitivity after serum administration. It is not stated whether the two patients who had immediate generalized reactions to the tests had previously had serum.

Immunity to Tetanus Induced by Combined Alum-Precipitated Diphtheria and Tetanus Toxoids. Peshkin, M. M.: Am. J. Dis. Child. **62**: 309, 1941.

The response of sixty-five allergic children who were given a third dose of combined diphtheria-tetanus alum toxoid three to fifteen months after the second dose was studied. While all children had not reached an adequate antitoxin titer (assumed to be 0.01 unit or more per cubic centimeter of serum) after the second dose, there were no such failures after the third dose. The height of the response after the third dose was usually directly related to the height of the response after the second dose. In other words, those who responded best after the second dose as regards antitoxin formation usually responded best after the third dose. All doses were 0.5 c.c. No asthma, urticaria, or other allergic reactions were encountered. Scratch tests done with the undiluted toxoids on thirty-six children in the group up to two years after the third dose were all negative.

Circumscribed Edema in the Nursling. Zerbino, V.: Arch. de pediat. d. Uruguay **12**: 354, 1941.

Four cases of Quincke's edema are reported in infants. Three were associated with such allergic manifestations as asthma, eczema, prurigo, and vomiting. Two reacted to egg white clinically as well as by skin test, while one gave a marked reaction to mother's milk intradermally.

The author points out that some instances of sudden death, laryngeal stridor, diarrhea, intestinal obstruction, colic, and convulsions may be due to visceral edema without cutaneous edema.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Vasomotor Rhinitis: Its Causation and Treatment. Stovin, Joseph S.: Arch. Otolaryng. **34**: 736, 1941.

In discussing the causation and treatment of vasomotor rhinitis, Stovin classifies this condition from the standpoint of diagnosis and treatment into the acute

and chronic types. The acute type includes all of those cases in which there is a demonstrable specific inhalant, such as in hay fever and rose fever. He states that in the chronic type one is usually unable to demonstrate hypersensitiveness to any specific irritating allergen. From the standpoint of general treatment he discusses such phases as psychotherapy, glandular therapy, drug therapy, and diet. Under local nasal treatment he recommends zinc ionization, application of phenol, electrocoagulation, irradiation, and the injection of alcohol into the nasal ganglion.

Roentgen Therapy for Bronchiectasis. Carpenter, Ralph C., Kerr, H. Dabney, and McMurray, J. S.: Arch. Otolaryng. **34**: 913, 1941.

Carpenter, Kerr, and McMurray report their observations on the treatment of thirty-eight patients with bronchiectasis by roentgen rays. The patients treated were divided into four groups according to the severity of the disease. The greatest improvement was seen in patients with minimal and moderate involvement. It was concluded that roentgen irradiation was only a moderately satisfactory treatment of bronchiectasis. This type of treatment resulted in some symptomatic improvement in about 50 per cent of those in whom the disease was mild or moderately advanced and a small percentage of those in whom the disease was far advanced. Finally this treatment is recommended as a valuable adjunct for those patients who have had little improvement from removal of the foci of infection by bronchoscopic drainage and other forms of therapy and who are not amenable to surgical treatment.

Management of Sinusitis in Cases of Bronchiectasis. Goodale, Robert L.: Arch. Otolaryng. **34**: 792, 1941.

Goodale reports his observations on the study of 150 cases of bronchiectasis observed at the pulmonary clinic of the Massachusetts General Hospital. He stated that over 90 per cent of the patients had sinusitis in some degree. In the series of cases studied it was noted that bronchiectasis had developed in one-third of the patients before the age of 10. They gave a history of pneumonia, pleurisy, whooping cough, or bronchitis as the direct precursor of the disease. There was another group in which the patients had endured bronchiectasis and sinusitis well past middle age. A third group was considered separately and consisted of young adults and adolescents. Among these patients radical surgery was considered for those who were believed to be able to stand the operation. It was stated that the infected sinus and infected chest can be considered as two separate foci of infection, which together constitute the patient's disability. Removal of infection from the one without removal of infection from the other was considered an incomplete operative procedure.

Etiology of Bronchiectasis. Schenck, Harry P.: Arch. Otolaryng. **34**: 958, 1941.

In discussing the matter of etiology of bronchiectasis, Schenck states that the concept that sinus infection is primary and bronchial infection secondary is supported by experimental evidence. He discusses the various routes of infection existing between the sinuses and lungs. In a series of experiments conducted it was shown that infectious material is readily transported from the upper to the lower respiratory tract and vice versa. Schenck finally concludes that, from the practical standpoint, it is obvious that the treatment of chronic suppurative disease of the lower respiratory tract is doomed to failure if it does not include the thorough treatment of sinus infection. Cure of the diseased sinuses will not always be followed by the arrest of the pulmonary lesions, and, once the bronchial disease is well established, eradication of sinus infection will not arrest the disease.

Massive Doses of Vitamins A and D in the Prevention of the Common Cold.
Spiesman, Irwin G.: Arch. Otolaryng. 34: 787, 1941.

Spiesman reports his observations on the uses of massive doses of vitamins A and D in the prevention of the common cold. It was noted that vitamins A and D in massive doses did not produce immunity to the common cold when given separately. When massive doses of these vitamins, however, were given together, 80 per cent of the subjects showed a significant reduction in both the severity and number of colds per year. The number of colds per year dropped to three, and the average duration was five days, with but little elevation in temperature. It is not assumed that the use of vitamins in the treatment of the common infectious cold was offered as a panacea. It is emphasized that average susceptibility must be taken into consideration, as well as the emotional state of the subject as affected by any innately unstable vasomotor mechanism.

Correspondence

The Editor,
THE JOURNAL OF ALLERGY.

I have recently found a useful mask for hay fever patients who have been unable to take their injections or for those who have to work about a farm and expose themselves to high densities of many kinds of dust.

Fig. 1.

The No. 5 Bantam Light Weight Respirator (Fig. 1) sold by W. S. Willson Corporation, 123 Varick Street, New York City, has met the unqualified approval of a number of patients. It costs less than $2.50, weighs less than 4 ounces, and offers no hindrance to respiration. The No. 5 respirator which is illustrated was originally designed for pneumoconiosis-producing dusts and mists. However, it has apparently proved effective against the entrance of pollen grains into the nose and lungs. It offers no protection to the eyes. I am not suggesting that this respirator be used to replace the conventional form of vaccine therapy, but I do believe it will prove of great service whether patients have had injections or not if they are necessarily exposed to large quantities of pollen or other dust. It has been most useful during the height of the pollen season when worn several hours during the day.

HAROLD A. ABRAMSON.

Nov. 7, 1941

The Editor,
THE JOURNAL OF ALLERGY.

The article by Dr. Ralph I. Alford in the September JOURNAL OF ALLERGY is presented as a "simple and improved procedure." The method, in brief, consists in the use of the Rheinberg filters for obtaining color contrast and an extra ocular and extension tube for obtaining greater detail.

Without question the Rheinberg filters produce a high degree of color contrast, but *only* between the background and the objects upon it. On an unstained atmospheric sample viewed by the usual method, pollens and fungus spores stand out from other particles by their own color and may be identified by their own different characteristic shades and densities. The Rheinberg method destroys all these distinctions and imparts the same high color to all pollens, spores and dirt particles. The method is thus essentially a dark field method by which many of the details easily seen by transmitted light are lost altogether.

In order to utilize the color effect of the Rheinberg filters to the fullest extent it is necessary to use the objective at its fullest aperture. This procedure introduces the following optical difficulties:

1. An image formed by an objective operating at full aperture is not an optimum image from the viewpoint of definition because the optical correction of the ordinary dry achromatic objective, and to a certain extent even the apochromats, is such that the objective gives its best performance when used at three-fourths of its full aperture. Unless an objective is corrected for use without a cover glass further aberrations are introduced—especially if the objective is used at its full aperture.

2. An image formed by an objective operating at full aperture is not an optimum image with regard to depth of focus, since the depth of focus is inversely proportional to the aperture at which an objective is used.

I agree that a magnification of 100 diameters is hardly enough for routine pollen and fungus spore counting, and that in seeking a satisfactory increase in magnification we should not discard the low power (10×) objective. The limit of useful magnification for a 10× objective (usual numerical aperture 0.25) is rated at slightly in excess of 250. Any magnification with such an objective above 250 is regarded as empty magnification. Long experience in pollen counting has led me to adopt 150 as a satisfactory magnification. This is obtained by using a 15× ocular. Other workers may find that 200 or even 250 is better, but it must be remembered that any increase in magnification decreases the width of the field which can be examined, thus increasing the necessary coverage in counting a unit area.

The addition of an extension tube and an extra ocular, as suggested by Dr. Alford, is only a makeshift scheme for securing a relatively small increase in magnification, which may be more easily secured by

the purchase of an inexpensive high powered eyepiece of 25×. The important point is that the device does not result in the excessive empty magnification of 750 as calculated by the author, but only approximately 250 as can be proved by direct measurement of the resulting image. A final minor objection is that the added ocular results in a reversed image.

In my opinion the utmost simplicity in routine pollen counting of oil-coated slides is attained by the use of a standard microscope with a 15× ocular used with a 10× objective. When occasional greater detail is needed, it can easily be obtained by change of temporary objectives or the use of a rapid-acting stain, such as Calberla's solution.

O. C. DURHAM.

Nov. 27, 1941

Errata

In the September issue of the JOURNAL in the article "The Absorption of Injected Pollen Antigens" by Samuel J. Levin and Louis E. Heideman, in Table I, column 4, page 619, the heading "Minutes after ragweed extract given intramuscularly" should read: "Minutes after ragweed extract given subcutaneously," and in column 5 the heading "Minutes after timothy extract given subcutaneously" should read: "Minutes after timothy extract given intramuscularly."

In the November issue of the JOURNAL in the article "Serologic Changes After Oral Ragweed Pollen Therapy in Children" by Samuel J. Levin and Lillian Shulsky in Summary, paragraph 3, line 2, the phrase "2 grains a day" should read: "2 grains twice a day."

In the November issue in the heading on page 1 and in the footnotes throughout the JOURNAL the word Association instead of Society should be used in the title of the American Association for the Study of Allergy.